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ORIGINAL ARTICLE

pH sensitive polymer nanoassemblies based on cyclodextrin polymer

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Abstract In this work, amphiphilic pH sensitive dextrans have been designed to elaborate complex systems with β-cyclodextrin polymers. The synthesized di-substituted dextrans bear purely hydrophobic groups (either dodecyl, C12, or adamantyl, Ada) and cyclohexancarboxylic acid groups (CHX). The assemblies formed by simply mixing the di-substituted dextrans and pβCD solutions show interesting pH dependence. At pH 7 (CHX groups fully charged), the polymers mixture leads to monophasic solutions; at pH 2 (CHX groups fully protonated), the polymers mixture leads to associative phase separations with precipitation. Between these two limiting cases (at pH around 4), associative phase separation still occurs but leads to the formation of metastable nanoassemblies.

Keywords Cyclodextrin polymer · Amphiphilic dextran · Nanoassembly · pH sensitive

Introduction

Polymer nanoassemblies are attracting increasing interests in the fields of biomedical and biotechnological applications, e.g., drug delivery [1, 2]. Recently, we have shown that polymer nanoassemblies may be obtained by simple mixing in pure water of two neutral and hydrophilic polymers [3]. The first polymer is a β-cyclodextrin polymer (pβCD) which interacts via a "lock and key" mechanism with the second one, a hydrophobically modified dextran (MD) bearing low amounts of dodecyl moieties (typically 4–6 mol%). In precise concentration conditions (<5 g L^{-1}), the spontaneous association of the two polymers led to compact nanosized structures. Very promising results related to drug delivery applications using these nanoassemblies have also been obtained [4]. However, these systems present a major drawback which is their sensitivity to ionic strength: the nanoassemblies are destabilized by salt addition even at very low concentration.

The aim of this work is to elaborate a system based on similar nanostructures but involving more complex interaction mechanisms in order to tune their properties and reach the required stability and sensitivity to environmental parameters for biomedical applications. Di-substituted dextrans have thus been considered: one group is simply hydrophobic (adamantyl, Ada, or dodecyl group, C_{12}) and the other one (cyclohexancarboxylic acid group, CHX) combines a pH-dependent affinity for the cyclodextrin (CD) cavity and pH-dependent charge density bringing electrostatic interactions in the system. In this paper, we briefly report the influence of this new moiety on the association properties of MD with pβCD, and determine the conditions in which stable nanoassemblies could be formed. The nanoassemblies are characterized by dynamic light scattering, zeta potential, transmission electronic microscopy and isothermal titration microcalorimetry.

Experimental part

The synthesis and the characterization of pβCD and MDs have already been described [5]. The pβCD sample presents a CD content (g/g), a weight average molecular weight M_w and a polymolecularity index of 63% (g/g), 2.6×10^6 g mol^{-1} and 4.9, respectively. The MDs are all

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Table 1 Name and composition of the different MDs

Name	CHX group %	C_{12} or Ada group %
MDCHX	4.2	
MDC_{12}		6.9
MDAda		5.9
MDC_{12}-CHXa	6.6	6.3
MDC_{12}-CHXb	2.7	6.9
MDAda-CHX	2.3	5.9

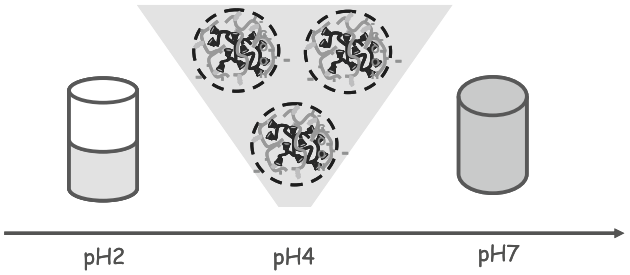

Fig. 1 Schematic behaviour for the di-substituted MD and pβCD mixture at different pH

prepared from the same precursor dextran of molecular weight 4.3×10^4 g mol^{-1}, except for MDCHX which is prepared from a precursor dextran of molecular weight 9.9 10^4 g mol^{-1}.The name and the degree of substitution of MDs are reported in Table 1.

Nanoassemblies are obtained by mixing 0.6 mL of MD and 0.6 mL of pβCD solutions (prepared one day before) at room temperature under magnetic stirring at 150 rpm. The concentration of both solutions is fixed at 1 g L^{-1}, their pH is adjusted before mixing at the chosen value by addition of HCl or NaOH.

The mean hydrodynamic diameter and the polydispersity index (PdI) of the nanoassemblies are determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Model ZEN3500) from Malvern Instrument equipped with a He–Ne laser (the zeta potential measurements are carried out using the same apparatus). Isothermal titration microcalorimetry (ITC) is made using a MicroCal VP-ITC microcalorimeter at 25 °C, pβCD solutions (10^{-2} mol L^{-1}) used as titrants and MD solutions (0.5–1.4 10^{-3} mol L^{-1} in hydrophobic groups) as analytes. Transmission Electron Microscopy (TEM) observations are conducted on a Philips Tecnai F20 ST microscope (field-emission gun operated at 3.8 kV extraction voltage) operating at an acceleration voltage of 200 kV. The samples are deposited on a 400 mesh carbon copper grids that have been previously treated by air plasma.

Results and discussion

In the case of MDC_{12} and pβCD in pure water, phase diagram studies have shown that the two polymers are subject to associative phase separation [3]. At low concentrations (<5 g L^{-1}), the mixtures are in the biphasic domain but no macroscopic phase separation is observed: they present a Tyndall effect and metastable nanoassemblies are formed with a spherical shape and a mean diameter around 200 nm. In the case of di-substituted dextrans, mixing of MDs and pβCD solutions at low concentration (<5 g L^{-1}) leads to very different results depending on pH, as schematically presented in Fig. 1.

It should be pointed out that all the results described below were obtained for nanoassemblies prepared at a total concentration of 1 g L^{-1} and at a polymer weight ratio of 1/1.

At pH 2, one observes a very fast precipitation of the mixture while at pH 7, a clear solution is obtained. The di-substituted dextrans are fully protonated at pH 2, and acidifying the solution leads to an increase of the ionic strength. As in the case of MDC_{12}/pβCD and MDAda/pβCD nanoassemblies formed in pure water, increasing the ionic strength (addition of salt or pH adjustment) destabilizes the nanoassemblies and leads to precipitation of the mixture.

At pH 7, the anionic charge on the di-substituted dextrans brought by the carboxylate functions avoids precipitation of the mixtures (on the contrary, mixing of MDC_{12} and pβCD solutions at pH 7 leads to precipitation). The di-substituted dextrans interact with pβCD (association between the polymers is demonstrated by the ITC measurements, see below) and form soluble complexes. Analysis of these clear mixtures by dynamic light scattering shows a broad intensity distribution with a fast mode corresponding to the scattering of a small proportion of free chains (either MDs and pβCD chains) and a slow mode corresponding to the scattering from the soluble complexes (diameters around 70–100 nm). Few large size aggregates are also often observed (sizes larger than 1 μm).

The intermediate pH value, pH 4, corresponds to the situation where the charge degree of the di-substituted dextrans reaches almost 50% since the pKa are close to 4.5 [5]. The mixtures present a Tyndall effect, indicating the formation of nanoassemblies as reported in the case of MDC_{12}/pβCD in pure water [3]. Analysis of the samples by dynamic light scattering shows a mono-modal intensity distribution. The high intensity of the signal, more than 10 times larger than at pH 7, and the low polydispersity index (<0.2) reflect the compact structure of the nanoassemblies. In addition, the nanoassemblies can be separated by centrifugation (15000 × g, 30 min). Around 50% in weight of the polymers are recovered after removing the supernatant and drying the settled down nanoassemblies whereas the

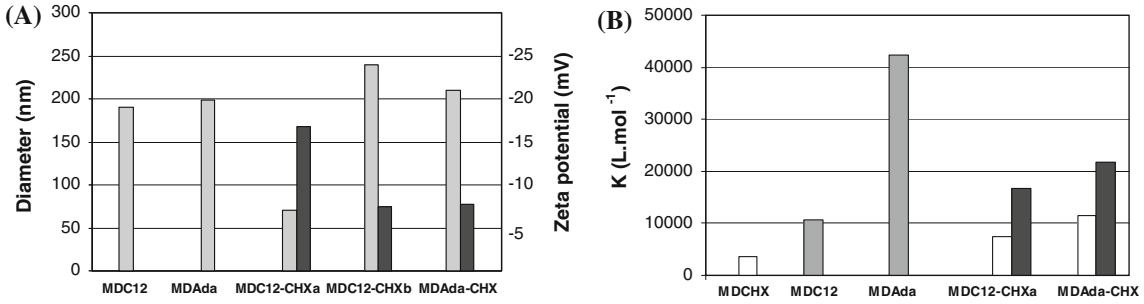

Fig. 2 **a** Mean diameters (*light shaded*) and zeta potentials (*dark shaded*) of the nanoassemblies formed at 1 g L^{-1}. **b** Binding constants of the different MD with pβCD at pH 4 (*unshaded*), pH 7 (*dark shaded*) and in pure water (*light shaded*)

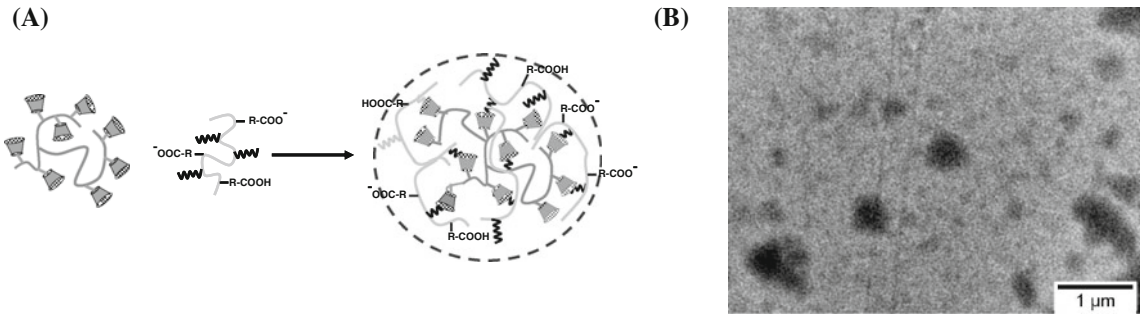

Fig. 3 **a** Scheme of the nanoassemblies formation at pH 4. **b** Transmission electron micrographs of MDC_{12}-CHXa/pβCD nanoassemblies

pH7 solutions did not show any settled down under the same conditions.

Figure 2a gives the mean diameter and the zeta potential of the nanoassemblies. Only the mean diameter obtained in the case of MDC_{12}-CHXa/pβCD is lower than the others, around 100 nm compared to 200 nm for all the nanoassemblies. The size decrease for MDC_{12}-CHXa/pβCD is certainly due to the charge of these nanoassemblies which leads to electrostatic repulsions and therefore to stabilization with less polymer chains involved than in the neutral case. The other di-substituted MD, MDC_{12}-CHXb and MDAda-CHX bear 2 times less charges than the previous one and give rise to nanoassemblies of comparable size as with MDC_{12} and MDAda. Concomitantly, the zeta potentials (ZP) evolve in the inverse order. ZP of MDC_{12}-CHXa/pβCD is almost two times higher, around −17 mV, than the ZP of −7.5 mV determined for MDC_{12}-CHXb/pβCD and MDAda-CHX/pβCD. These findings support the preferential localization of the negatively charged dextrans at the surface of the nanoassemblies, leading to their stabilization at an intermediate pH (pH 4). Decreasing the pH should decrease the charge degree of the MD and thus destabilize the nanoassemblies by reducing the electrostatic repulsions. On the contrary, increasing the pH should increase the charge degree and lead to soluble complexes. Figure 3a illustrates this mechanism of stabilization at pH 4.

Additionally, the nanoassemblies formed in the case of MDC_{12}-CHXa/pβCD at pH 4 have been observed by TEM. Figure 3b shows one of the obtained micrographs which emphasizes the spherical shape of the nanoassemblies. Various dark circular objects are observed, with diameters in the range of 100–500 nm. These diameters are in average larger than the previous ones determined by dynamic light scattering in the aqueous suspensions, probably due to partial collapse of the nanoassemblies over the grid after water drying.

The thermodynamic properties of the interactions between the different MDs and pβCD have been investigated by isothermal titration microcalorimetry (ITC). Both the processes of soluble complexes formation (in the case of the di-substituted dextrans at pH 7) and nanoassemblies formation (in the case of the di-substituted dextrans at pH 4) are exothermic. The experimental data are fitted with a theoretical curve assuming that each hydrophobic group grafted on dextran acts independently from each other and form 1/1 inclusion complexes with CD cavities. The enthalpy variations are comprised between 6 and 20 kJ mol^{-1}, absolute values lower than the ones obtained between MDs and βCD [5]. Moreover, positive entropy variations are observed in all the cases (TΔS comprised between 6 and 18 kJ mol^{-1}) whereas more defavorable values were obtained in the case of MD [5]. This could be attributed to cooperative polymer polymer interactions.

The nanoassemblies formation in the case of MDC_{12} and MDC_{12}-CHXa is mainly entropy driven ($T\Delta S > |\Delta H|$).

The binding constants, K, vary from 3.6×10^3 to 4.2×10^4 L mol^{-1} as shown Fig. 2b. For the mono-substituted dextrans, the highest binding constant is obtained for MDAda, as expected since the adamantyl group precisely matches in size and shape the CD cavity. In the case of the di-substituted dextrans, the dextran chains bear two kinds of hydrophobic groups with low affinity (CHX) or higher affinity (C_{12} or Ada) for the CD cavities. It was expected that the experimental results could be fitted with two well defined binding constants. Instead of that, the results are nicely fitted with one apparent binding constant. The values are higher than the one of MDCHX, but lower than the ones of MDC_{12} and MDAda (except in the case of MDC_{12}-CHXa at pH 7). Surprisingly, the apparent binding constants are higher at pH 7 than at pH 4. At pH 7, only the C_{12} or Ada groups participate to the binding process because the CHX groups are fully charged and show a very low affinity for the CD cavity [6]. On the contrary, the CHX groups are partly charged at pH 4 and participate to the binding, decreasing the value of the apparent binding constants compared to the pH 7 values.

Conclusion

In this work we have designed macromolecular assemblies with tuned electrostatic and host–guest interactions. Mixing in aqueous solution a cyclodextrin polymer and an amphiphilic di-substituted dextran generates pH-dependent associative phase separations with colloidal nanoassemblies formed in a well defined pH range. Such a design of stimuli responsive systems can be adapted for biomedical applications.

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