Tailorable Polymeric Assemblies Based on Host/Guest Interactions Between Modified Dextrans

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Summary: Nanoassemblies are spontaneously formed in dilute aqueous solutions by mixing of two dextrans modified with lauroyl and β-cyclodextrin moieties, respectively. This association process is mainly driven by host/guest interactions between the two polymers. The nanoassemblies' size depends on the substitution degrees of the polymers, and on the total concentration and composition of the mixtures. The results are compared to those previously obtained with adamantyl grafted dextrans. The number and the strength of the individual guest-host complexes between the two grafted dextrans is the key parameter to obtain small and stable nanoassemblies.

Keywords: associative polymers; cyclodextrins; host-guest systems; modified dextrans; nanoparticles; polysaccharides

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides constituted of 6, 7 or 8 glucose units forming ring-shaped molecules with a hydrophilic outer surface and a relatively hydrophobic internal cavity.[1] They form inclusion complexes with a large variety of hydrophobic guest molecules,[2] often forming complexes with improved solubility, and physical and chemical stability, making CDs very attractive drug carriers.[3]

Polymeric CD derivatives combine the inclusion properties of CDs and properties of polymers such as high molecular weight and high solubility. They are known to form gels or nanoparticles under defined conditions. Recent reviews report host-guest polymer assemblies with promising biomedical applications.[4–6]

Dextrans (DTs) are natural glucose polymer linked by α-1,4-glycosidic bonds, extensively used in biomedical science due to their biocompatibility and low toxicity. Recently, we reported the synthesis of βCD grafted DTs.[7] These well-defined polymers were prepared by "click" grafting βCD units onto DT chains, different spacer arms ensuring flexibility and accessibility for host-guest associations. Their excellent binding properties were demonstrated by isothermal titration microcalorimetry (ITC) with 2-(1-adamantyl)ethyl trimethylammonium bromide (Ada2) as a guest (association constants $K \sim 2\ 10^5\ L\,mol^{-1}$).

Our group also showed that, at low concentration ($<5\ g\,L^{-1}$), these polymers can lead to stable nanoassemblies by simply mixing with adamantyl (Ada) grafted DTs.[8] The two polymers interact via a "lock and key" mechanism, and the nanoassemblies have tailorable sizes, depending not only on the βCD and Ada substitution degree but also on the total concentration and composition of the mixtures. The results could be rationalized assuming a core/shell structure of the nanoassemblies, the core resulting from an associative phase separation of the two polymers stabilized by an external shell made of Ada grafted DTs and containing ions adsorbed from the solution.

The main objective of the present work is to study the influence of the host-guest

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interaction mechanism by changing the nature of the guest: lauroyl (C12) groups instead of adamantyl groups in the previous work.[8] Thus a series of C12 grafted DTs of varying substitution degree have been synthesized and the properties of the new nanoassemblies are compared to the ones obtained with Ada grafted DTs. It is already known that C12 grafted DTs self-associate in aqueous solution,[9] forming hydrophobic domains above a critical aggregation concentration (cac around $1\,g\,L^{-1}$ for a C12 molar substitution degree around 4%). This self-association does not occur with Ada grafted DTs and this could have an influence on the C12-based nanoassemblies' properties. C12 grafted DTs were already used to form nanoassemblies by mixing with high molecular weight βCD/epichlorohydrin copolymers.[10–13] These βCD copolymers and the ones we used in this work differ by their structure (branched and compact compared to linear structure, respectively) and their binding properties (with Ada2, around $5\ 10^3\,L\,mol^{-1}$[14] compared to $10^5\,L\,mol^{-1}$[7], respectively). In this work, we determine the thermodynamic parameters of nanoassemblies' formation by ITC, and the nanoassemblies' size and stability are studied as a function of the molar substitution degree, concentration and composition of the mixtures.

Materials and Methods

Materials

The different dextrans, D40, D70 and D110 (Dextran T40, T70 and T110, Pharmacosmos A/S, Denmark) with normative molecular weight of 40, 70 and 110 $10^4\,g\,mol^{-1}$, respectively, were dried overnight under vacuum at 115 °C before use. The synthesis of lauroyl substituted DTs was described for D40.[9] The same procedure was used to modify D70 and D110 and the substitution degree of the different modified DTs is reported in Table 1. The substitution degree was obtained by ^{1}H NMR in deuterated DMSO from the ratio of the integration of 21 lauroyl protons (0.9–2.4 ppm) and of the integration of the anomeric and hydroxylic protons (4.4–5.0 ppm). The synthesis of the βCD grafted DTs was recently reported[7] with their characterization and the substitution degree is given in Table 1 (determined by ^{1}H NMR in deuterated water).

Table 1.
Composition of the different C12 and βCD grafted DTs.

Compound	C12 molar %	Compound	βCD molar %
D40C12-3	2.9	D70GPβCD1	4.8
D40C12-5	4.7	D70GPβCD2	7.0
D40C12-6	6.1	D70GPβCD3	8.4
D40C12-9	9.1	D70GPβCD4	10.3
D70C12-6	5.7	D70GPβCD5	12.7
D110C12-6	6.3	D70GPβCD6	16.8

Isothermal Titration Microcalorimetry

Isothermal titration microcalorimetry (ITC) was carried out using a MicroCal VP-ITC microcalorimeter. In each titration, injections of 10 μl of βCD or βCD polymer solution (~5 to $8 \cdot 10^{-3}\,mol\,L^{-1}$ in cavities) were added from the computer controlled 285 μl microsyringe at an interval of 180 sec into the cell (volume of 1.4569 mL) containing the C12 modified dextran solution ($\sim 5 \cdot 10^{-4}\,mol\,L^{-1}$ in C12 groups) while stirring at 450 rpm. The experiments were carried out at 25 °C. The raw experimental data were obtained as the amount of heat produced per second following each injection of βCD or βCD polymer as a function of time. Integration of the heat flow peaks by the instrument software (after taking into account heat of dilution) gives the amount of heat produced per injection. The experimental data are fitted with a theoretical titration curve assuming a 1:1 model; the analysis is done using Origin with the software developed by MicroCal. The first point of the titration curve is disregarded as some liquid mixing near the tip of the injection needle takes place at the beginning of each ITC run. The enthalpy change, ΔH, the association constant, K, and the overall stoichiometry, n (syringe concentration over cell concentra-

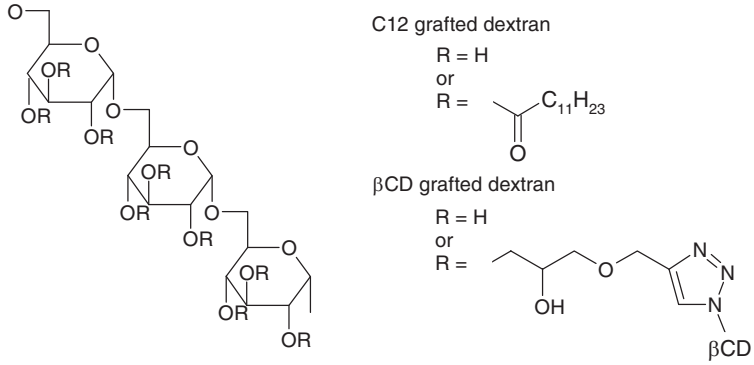

Figure 1.
Structure of the C12 and βCD grafted DTs.

tion), are the fitting parameters. The titrations were repeated at least two times, and the deviation of the calculated binding constants and enthalpy variation are less than 10 and 5%, respectively.

Nanoassemblies Preparation and Characterization

All polymer solutions were prepared at least one day before the different experiments in order to equilibrate the samples. The concentration of the different polymers solutions was fixed in order to have the same concentration of C12 groups or βCD cavities ($2.25\ 10^{-4}$ or $4.5 \cdot 10^{-4}$ mol L^{-1}). Nanoassemblies were prepared by mixing modified DTs and βCD copolymers solutions at room temperature under magnetic stirring at 150 rpm. The final molar C12:βCD ratio was obtained by varying the volume of each solution. The mean hydrodynamic diameter and the polydispersity index (PdI) of the nanoassemblies were determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Model ZEN3500) from Malvern Instrument equipped with a He-Ne laser ($\lambda = 633$ nm, scattering angle 173 °). Each sample was measured ten times for ten seconds at 25 °C and the measurements were made in duplicate; the standard deviation is around 2%. The reported mean value (or Z-average size) and the PdI are obtained by cumulant analysis (fit of the logarithm of the correlation function by a 3rd order polynomial) and they are the average of at least three different experiments.

Results

Structure of the different dextrans is given in Figure 1 and their name and composition are reported in Table 1.

The different C12 modified DTs were obtained by an esterification reaction between the commercial dextrans and lauroyl chloride in DMF. The βCD grafted DTs used in this work were synthesized by ''click'' chemistry using alkyne modified D70 DTs onto which monoazido βCD was grafted.[7]

The interactions of several D40C12 with β-CD were studied by isothermal titration microcalorimetry (ITC). The process is exothermic and the different thermodynamic parameters are reported in Table 2. The formation of complexes is an enthalpy-driven process ($\Delta H < 0$) with an entropic contribution ($T\Delta S > 0$) and the binding constant K decreases with increasing D40C12 substitution degree. At the concentrations used in ITC experiments, all D40C12 are at higher concentration than their cac. For several D40C12, cac values comprised between 0.3 and 2.2 g L^{-1} ($\sim 10^{-4}$ to $4\ 10^{-4}$ mol L^{-1} in C12 groups) were determined by fluorescence using pyrene as a probe.[9] Obviously, this self-

Table 2.
Thermodynamics parameters at 25 °C for inclusion complex formation of D40C12 with βCD and for the nanoassembly formation of D40C12 with D70GPβCD.

Guest	Host	10^{-3}*K L/mol	ΔG kJ/mol	ΔH kJ/mol	TΔS kJ/mol
D40C12-3	βCD	5.5	−21.3	−16.1	5.2
D40C12-5	βCD	2.6	−19.5	−16.8	2.7
D40C12-6	βCD	1.4	−17.9	−17.2	0.7
D40C12-3	D70GPβCD5	45.3	−26.6	−23.1	3.5
D40C12-5	D70GPβCD5	43.8	−26.4	−23.5	2.9
D40C12-6	D70GPβCD4	44.1	−26.5	−24.8	1.7
D40C12-6	D70GPβCD5	41.8	−26.3	−23.8	2.5
D40C12-6	D70GPβCD6	54.4	−30.1	−25.7	4.4

association mechanism competes with the formation of inclusion complexes. This explains the reported decrease of the binding constants when the D40C12 concentration is over the cac.[13] The obtained K values (1–5 10^3 L mol^{-1}) are in agreement with the ones previously determined either by ITC[9,11,13] or by fluorimetric titration[9].

Interestingly, a large increase of at least one order of magnitude ($K \sim 4$–5 10^4 L mol^{-1}) is observed for the binding constants determined for the formation of nanoassemblies (Table 2). This might be explained by the proximity of the interacting species, one interaction favors another, leading to collective properties not present for the individual components. Such an increase has already been observed with D40Ada and D70GPβCD.[8] K slightly depends on the DTs' substitution degree; the maximum value is obtained for the complex formation between D40C12-6 and D70GPβCD6 having the highest C12 and βCD substitution, respectively. These K values are slightly higher than the ones determined for D40C12 and βCD/epichlorohydrin copolymers,[11,13] but one order of magnitude lower than the ones determined with D40Ada and D70GPβCD ($K \sim 3$–6 10^5 L mol^{-1}).[8] This might be partly related to the self-association of D40C12 under the conditions of the ITC experiments, decreasing the availability of the alkyl groups already structured in the hydrophobic domains. This will induce differences for the properties of the nanoassemblies (see below).

In all cases, the formation of the nanoassemblies is an enthalpy-driven process ($\Delta H < 0$) with a positive entropic contribution ($T\Delta S > 0$) as in the case of nanoassemblies formed between D40Ada and D70GPβCD.[8] The interactions are most likely driven by a range of energetic contributions, where van der Waals' forces may be prominent since the process is enthalpy-driven ($|\Delta H| > |T\Delta S|$).[15] This was quite different for the nanoassemblies formed with βCD/epichlorohydrin copolymers.[11,13] In this latest case, the process is entropy-driven ($|\Delta H| < |T\Delta S|$), and this is mainly related to the copolymer structure. βCDs are largely modified in βCD/epichlorohydrin copolymers due to multiple substitutions, leading to an expansion of the cavity size and an entropy increase.[2,13,15] D70GPβCD are well-defined polymers grafted with monosubstituted βCDs, the cavities being still largely accessible. The complex formation between D40C12 and βCD or D70GPβCD implies moderate positive variation of entropy.

In our previous work,[8] we have demonstrated that the nanoassemblies' properties largely depend on the Ada and βCD substitution degrees. Critical values of 4 and 6 mol%, respectively, have been determined, under which no assemblies are formed. Above these values, the number of links that can be formed through inclusion complexes between the dextran chains determines the nanoassemblies' size and stability. In addition, the high binding constants measured under the conditions of

nanoassembly formation, $K > 10^5\,L\,mol^{-1}$, reflect the strength of these links.

In a first set of experiments, we studied the nanoassemblies' properties formed between the different D40C12 and D70GPβCD grafted DTs at a 1:1 molar ratio C12:βCD (total concentration of 2.5 10^{-4} mol L^{-1}). No stable nanoassemblies are formed with D40C12-3, whatever the βCD substitution degree of D70GPβCD is. After 3 hours, only few large aggregates are present in the mixtures. Figure 2A shows the nanoassemblies diameter for the other pairs of dextrans.

One can notice that the diameter decreases with the increase of both C12 and βCD substitution degrees. For example, the average diameter decreases from 220 nm to 100 nm for D70GPβCD6 mixed with the different D40C12, for a C12 substitution degree varying between 4.7 and 9.1%. In the same way, the average diameter decreases from 430 nm to 100 nm for D40C12-9 mixed with the different D70GPβCD, for a βCD substitution degree varying between 4.8 and 16.8%. Increasing the substitution degree of both DTs increases the number of links that can be formed between the DTs chains through inclusion complex formation. This leads not only to smaller nanoassemblies, but also to more stable nanoassemblies. Therefore, at the lowest C12 and βCD substitution degrees (Figure 2A), the average nanoassembly diameter is already over 900 nm after 3 hours implying that the nanoassemblies are not stable. Figure 2B reports the diameter as a function of time for nanoassemblies formed between D40C12-6 and D70GPβCD3 or D70GPβCD5. This shows that the size increase over 4 days is larger for the nanoassemblies formed with D70GPβCD3 (substitution level of 8.4%) than with D70GPβCD5 (substitution level of 12.7%).

The molecular weight of the dextran chain has little or no influence on the size of the nanoassemblies. D40C12-6 and D70C12-6 behave almost identically, only D110C12-6 leads to smaller size of the nanoassemblies formed with the different D70GPβCD as shown on Figure 3A.

D110C12-6 exhibits a much higher number of alkyl groups per chain (∼ 43) than for the two other dextrans (∼ 15 and ∼ 25 for D40C12-6 and D70C12-6, respectively). On the other hand, excess of C12 groups leads to an increase of the average nanoassembly diameter. For example, the average diameters are 240 nm, 250 nm and

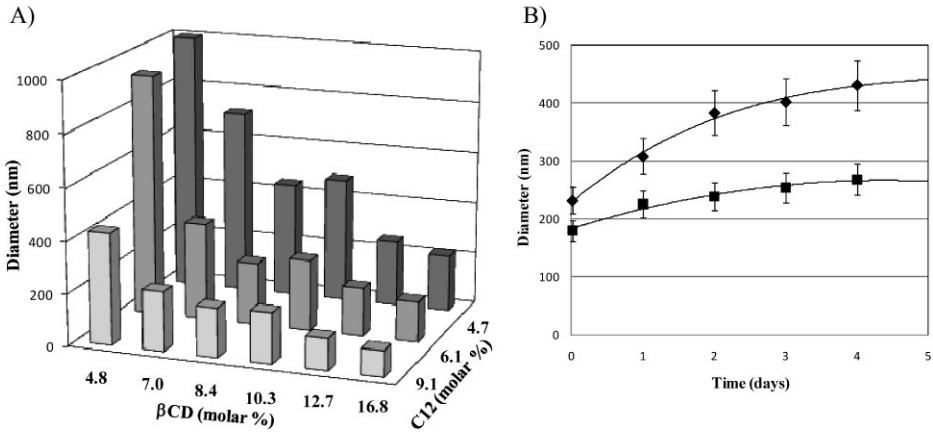

Figure 2.
A) Diameter of the nanoassemblies formed between the different D40C12 and D70GPβCD after 3 hours B) Diameter as a function of time for the nanoassemblies formed by mixing D40C12-6 with D70GPβCD3 (◆) or D70GPβCD5 (■) (total concentration, 2.2 10^{-4} mol L^{-1}).

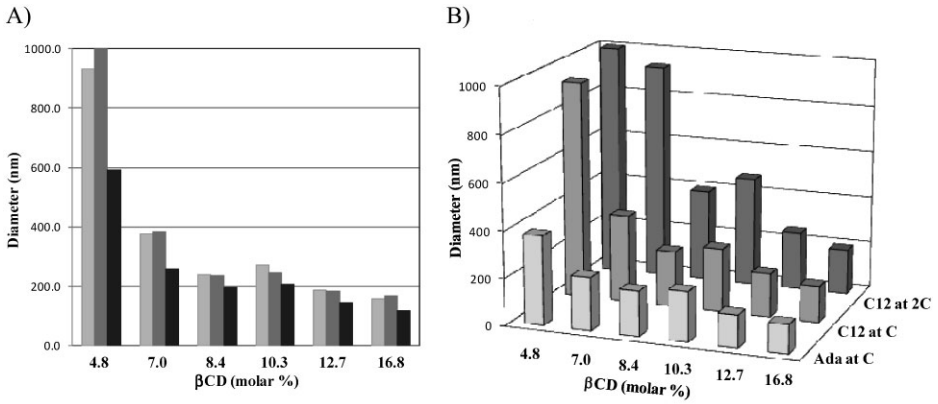

Figure 3.
Diameter of the nanoassemblies formed after 3 hours between the different D70GPβCD and A) D40C12-6 (▒), D70C12-6 (▒) and D110C12-6 (■) B) D40Ada6 (▒) D40C12-6 (▒) with a total concentration C = 2.2 10^{-4} mol L^{-1}, D40C12-6 (■) with a total concentration 2C.

320 nm for the nanoassemblies formed between D40C12-6 and D70GPβCD5 at molar ratios of 1:3, 1:1 and 3:1, respectively. For the systems showing a larger diameter at a 1:1 ratio (C12:βCD), excess of both C12 groups and βCD cavities destabilize the nanoassemblies. The average diameters are 515 nm, 395 nm and 655 nm for the nanoassemblies formed between D40C12-6 and D70GPβCD3 at molar ratios of 1:3, 1:1 and 3:1, respectively (total concentration of 4.5 10^{-4} mol L^{-1}).

All these results indicate that smaller and more stable nanoassemblies are obtained when the number of links is maximized, this being reached with higher substitution degrees and at a 1:1 molar ratio. This effect of the number of interaction points on the size and stability is quite similar to the one we described for series of Ada grafted DTs.[8] Anyhow, there are differences between nanoassemblies formed with C12 or Ada grafted DTs. Figure 3B shows the nanoassemblies' diameter formed between D70GPβCD and either D40C12-6 or an Ada grafted dextran, D40Ada6. D40Ada6 has a substitution degree of 6.6%, leading to nearly the same number of hydrophobic groups per chain as D40C12-6. One can notice that the nanoassemblies' diameter is always smaller for D40Ada6 than D40C12-6 with the same D70GPβCD. For example, the average diameters are 125 nm and 155 nm for D40Ada6 and D40C12-6 with D70GPβCD6, respectively. This effect is enhanced with D70GPβCD having lower βCD substitution degrees. The nanoassemblies formed between D70GPβCD1 and D40Ada6 have a diameter around 385 nm whereas the ones formed with D40C12-6 are larger and coalesce very rapidly. After 3 hours, the diameter is already over 900 nm. In addition, the size of the nanoassemblies formed with D40C12 is very sensitive to the total concentration. Figure 3B shows that increasing the total concentration by a factor 2 leads to coalescence of the nanoassemblies formed between D40C12-6 and D70GPβCD1 or D70GPβCD2 as the diameters are larger than 800 nm after 3 hours. Even with D70GPβCD6 having the highest βCD substitution degree, the diameter increases from 155 to 195 nm. In this concentration range, the size of the nanoassemblies formed with D40Ada was almost constant.[8] This demonstrates a lower stability of the nanoassemblies formed with C12 grafted DTs compared to Ada grafted DTs, and this is in agreement with the 10 times difference of the association constants determined by ITC.

Discussion and Conclusion

The results show critical values of C12 and βCD substitution degree (around 4 and 6 mol%, respectively), which are similar to those determined with D40Ada and the same βCD copolymers. Below these values, no nanoassemblies are formed; above these values, the diameter of the nanoassemblies decreases with the C12 and βCD substitution degrees. Anyhow, at same molar concentration and same substitution degree of hydrophobic groups, the nanoassemblies formed with D40C12 have always a larger diameter than the ones determined with D40Ada, the former being therefore less stable over time. Two main raisons could be put forward. In all these experiments, the concentration is over the D40C12 cac. During the nanoassemblies' formation, most of the hydrophobic domains are dissociated by the C12-βCD interactions. Nevertheless, it is reasonable to assume that few hydrophobic domains can be still present inside the nanoassemblies, leading to a lower number of links between grafted DTs. Additionally, lower association constants are obtained with D40C12 compared to D40Ada by ITC in the conditions of nanoassemblies' formation (adamantyl derivatives always exhibit higher association constants than dodecyl derivatives). This will notably decrease the strength of the interaction between the modified dextrans. Decreasing number and strength of the links increases the nanoassemblies' size and decreases their stability.

The core-shell model previously proposed for the nanoassemblies formed between Ada and βCD grafted DTs can also be used to rationalize the results obtained in this work. The core of the nanoassemblies should result from associative phase separation of the two DTs; it should also contain few hydrophobic domains. The external shell should be made of C12 grafted DTs, the C12 groups could anchor the shell to the core by making inclusion complexes with the core βCDs. The stability is afforded by electrostatic repulsion between the nanoassemblies due to ions adsorption from the solution during the phase separation process. A schematic structure of the nanoassemblies is given Figure 4.

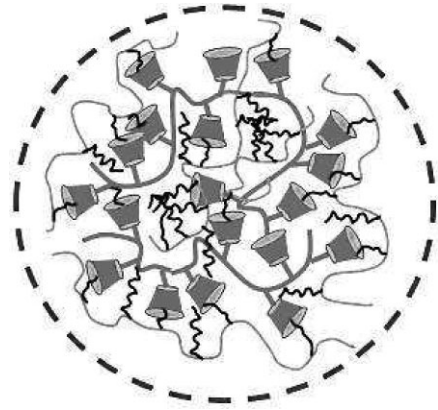

Figure 4.
Schematic structure of the nanoassemblies.

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