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Note

Influence of chemical structure of amphiphilic β-cyclodextrins on their ability to form stable nanoparticles

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Abstract

The aim of the study was to establish a correlation between the chemical structure of amphiphilic β-cyclodextrins (β-CDa) and their ability to form stable nanospheres. Amphiphilic derivatives were obtained according to an optimized well-known three-step synthesis. The selective acylation of the secondary face of β-CD being well controlled, several β-CDa presenting different substitution degree were synthesized. The self-organization properties of the derivatives were evaluated. The peracylated β-CD, i.e. bearing 14 alkyl chains on the secondary hydroxyl groups, does not yield stable nanosphere suspension, even in the presence of a non ionic surfactant in the aqueous phase. However, the presence of partially acylated derivatives within the β-CDa allows self-assembly into stable nanosphere suspension.

Keywords: Amphiphilic β-cyclodextrins; Chemical structure; Nanosphere suspension; Stability

In the last decade, a large number of chemical modifications on cyclodextrins have been investigated in order to extend the physico-chemical properties of these cyclic oligosaccharides (Wenz, 1994). Among these chemically modified cyclodextrins, amphiphilic derivatives especially β-cyclodextrins (β-CDs) bearing fatty acid chains on the secondary hydroxyl groups can self assembly yielding original and stable nanospheres (Wouessidjewe et al., 2000). In an attempt to elucidate the relationship between chemical structure of β-CDa and their ability to nanoassociate, the self-organization properties of different selectively acylated derivatives of β-CD were recently evaluated. First stability results are also presented.

The hexanoyl β-cyclodextrin esters (Fig. 1) were obtained by a synthetic route as recently described (Aous et al., 2001). The procedure implied (i) protection of β-CD primary hydroxyl groups, (ii) acylation of the secondary face via hexanoic chain grafting and (iii) deprotection of primary face. It

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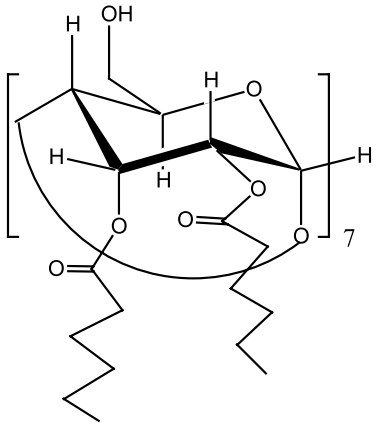

Fig. 1. General chemical structure of β-CDa.

has to be pointed out that the synthetic route was different from the one described previously (Zhang et al., 1991). Particularly, the acylating and deprotection conditions were modified. The derivatives were characterized by ^{13}C-NMR and ^{1}H-NMR as well as mass spectrometry using the matrix-assisted laser desorption/ionization-time of flight (Maldi-Tof) mode. Analyses were performed with a Perkin Elmer mass spectrometer (Applied Biosystems-Voyager Edit XL). Nanospheres were obtained by a nanoprecipitation method which consisted of injecting a 0.5-mg/ml acetone (Carlo Erba, Val de Reuil, France) solution of β-CDa (40 ml) into an aqueous phase (40 ml) under magnetic stirring. The aqueous phase (distilled-deionided water) was free of surfactant or was added to poloxamer 188 (Pluronic F68,

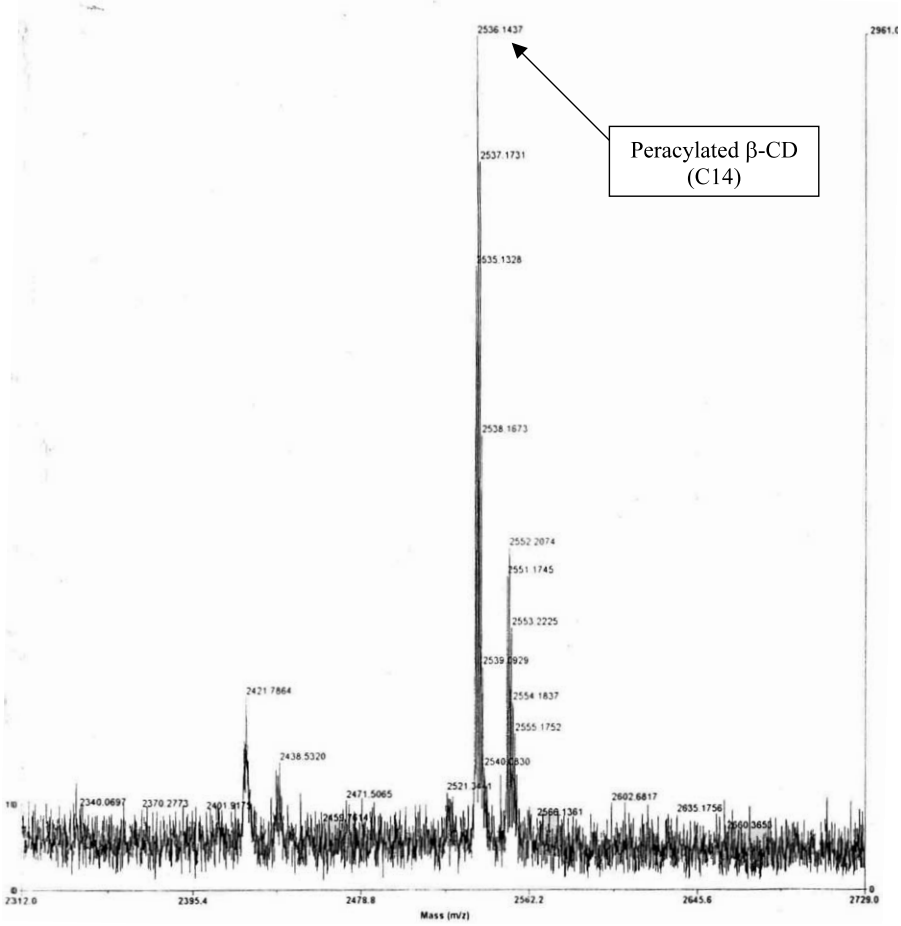

Fig. 2. Maldi-Tof spectrum of peracylated (2,3-di-*O*-hexanoyl)-β-cyclodextrin.

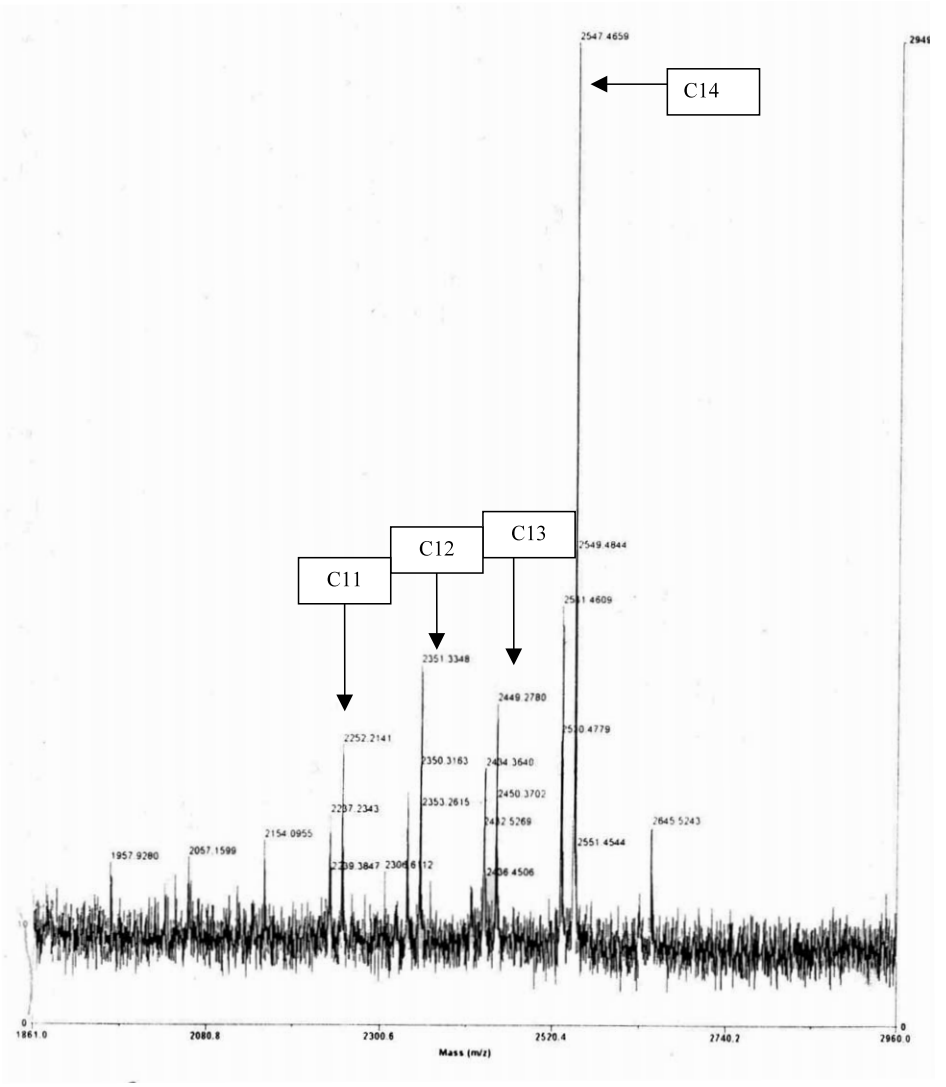

Fig. 3. Maldi-Tof spectrum of peracylated (2,3-di-*O*-hexanoyl)-β-cyclodextrin combined with differently acylated species.

BASF, Ludwigshafen, Germany) at 1 mg/ml concentration. Following nanoprecipitation, acetone was removed under reduced pressure and the suspension was concentrated to a final volume of 15 ml. Finally, the colloidal aqueous suspensions were filtered through a 0.8-μm (Millex AA, Millipore, France) and stored in closed vials at + 6 °C. Particle size measurements were performed by dynamic light scattering using a Zetasizer 3000 (Malvern Instruments, Malvern, UK). Analyses were carried out at an angle of 90°, at 22 °C after dilution of the samples in 0.1 μm filtered distilled water. A Phillips CM 200 'Cryo' microscope was used for transmission electron microscopy studies (TEM). Thin vitrified films of nanoparticle suspensions were prepared for cryomicroscopy using the Leica EM CPC fast freezing device (Putaux et al., 1999).

By varying the experimental conditions, two hexanoyl β-CD esters could be synthesized, showing distinct chemical structures. A peracylated derivative (2,3-di-*O*-hexanoyl)-β-cyclodextrin, i.e.

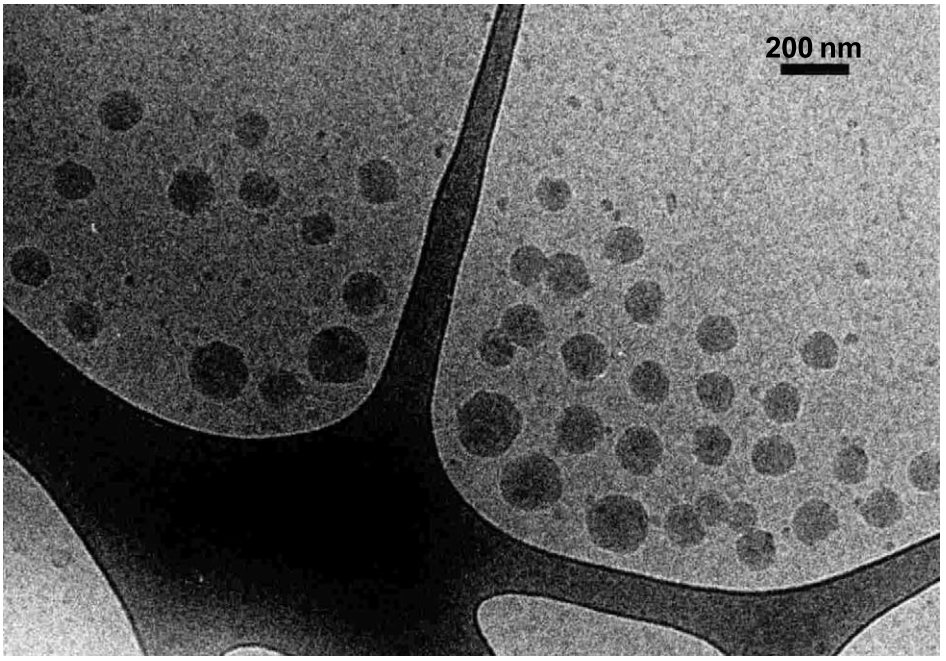

Fig. 4. Transmission electron cryomicrographs of nanoparticles.

containing fourteen acylated alkyl chains on the secondary hydroxyl groups, corresponding to m/z 2536 $[M + Na]^+$ in mass spectrum, was obtained (Fig. 2). The second product was a combination of peracylated derivative (m/z 2547 $[M + K]^+$) and small amounts of subacylated species bearing eleven to thirteen hexanoic chains on the secondary hydroxyl groups, corresponding respectively to m/z of 2252, 2351, 2449 $[M + K]^+$ (Fig. 3). It has to be noted that the Maldi-Tof mass spectrometry was essential to differentiate one derivative from the other. Indeed, the observed differences between the two compounds did not appear in NMR analyses, the partially acylated species being present at less than 5% in the mixture. For this reason, the paper deliberately presents the only mass spectra analyses.

The peracylated β-CD, i.e. bearing fourteen alkyl chains on the secondary hydroxyl groups, does not lead to stable nanosphere suspension, even in the presence of Pluronic F68, which was known to favor β-CDa nanosphere stabilization (Skiba et al., 1996). Despite the presence of the non ionic surfactant in the medium, a coalescence phenomenom of dispersed nanospheres was observed before the complete elimination of organic solvent from the suspension. At the opposite, the presence of partially acylated species in the product allowed self-assembly into 200 nm nanosphere suspension without the use of Pluronic F68 in the preparation medium (Fig. 4 and Table 1). These results indicated that the presence of species differently acylated, even in a small amount (lower than 5% in weight), was involved in the stabilization of suspended nanoparticles. As presented in Table 1, these suspensions displayed unchanged mean size after 7 months storage at $+6$ °C.

Table 1
Size measurements (intensity mode)

Time (months)	Hydrodynamic diameter (nm)	Polydispersity index
0	212 ± 198	0.17
7	211 ± 202	0.20

Analysis program: auto-Contin.

In conclusion, the ability of the β-CDa to nanoassociate was found to be related to the chemical structure of the derivatives. The control of the amount of partially acylated species in the amphiphilic β-CD may be an issue to regulate mean size diameter of nanospheres and therefore stability of the suspensions. This point is currently being looked at by our group.

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References

Aous, S., Baussanne, I., Gèze, A., Defaye, J., Wouessidjewe, D., 2001. Synthesis of amphiphilic cyclodextrins for nanoparticle preparation. 11th Carbohyd. Symp., Lisbon, p. 253.

Putaux, J.L., Buléon, A., Borsali, R., Chanzy, H., 1999. Ultrastructure aspects of phytoglycogen from cryo-transmission electron microscopy and quasi-elastic light scattering data. Int. J. Biol. Macromol. 26, 145–150.

Skiba, M., Duchêne, D., Puisieux, F., Wouessidjewe, D., 1996. Development of a new colloidal drug carrier from chemically-modified cyclodextrins: nanospheres and influence of physicochemical and technological factors on particle size. Int. J. Pharm. 129, 113–121.

Wenz, G., 1994. Cyclodextrins as building blocks for supramolecular structures and functional units. Angew. Chem. Int. Ed. Engl. 33, 803–822.

Wouessidjewe, D., Gèze, A., Aboubakar, M., 2000. Study of physical stability of amphiphilic β-cyclodextrin-based nanosphere suspension. Proceedings of the 3rd World Meeting APV/APGI, Berlin, pp. 459–460.

Zhang, P., Ling, C.C., Coleman, A., Parrot-Lopez, H., Galons, H., 1991. Formation of amphiphilic cyclodextrins via hydrophobic esterification at the secondary hydroxyl face. Tetrahedron Lett. 32, 2769–2770.