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ORIGINAL ARTICLE

Effect of β-cyclodextrin and its derivatives on caveolae disruption, relationships with their cholesterol extraction capacities

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Abstract Endothelial cells (HUVEC) were treated with β-cyclodextrin (β-CD) and hydroxypropylated or methylated derivatives solutions to confirm their lack of affinity with phospholipids and their specificity towards cholesterol. Further studies were performed on bovine aortic endothelial cells to assess the effect of β-CDs (mainly methylated derivatives) on membrane microdomains (lipid rafts or caveolae), by detecting the caveolae marker caveolin-1 in fractions of sucrose gradients. A displacement from the lighter to the heavier fractions, characteristic of caveolae disruption, was observed using CDs. The strongest effect was obtained with dimethyl-β-CD, for which an accumulation of caveolin-1 was observed in the bottom of the gradient. Crysmeb® and trimethyl-β-CD seemed to have the weaker effects as a significative amount of caveolin-1 was still detected in the light fraction corresponding to caveolae. β-CD and CDs having a degree of methylation a bit lower than 2 showed intermediate effects. The results of the present study on microdomains seem in good correlation with the cell cholesterol extraction capacities of CDs previously determined.

Keywords Cyclodextrins · Endothelial cells · Lipid rafts · Caveolae · Cholesterol · Phospholipids

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Introduction

For years, the fluid mosaic model of Singer and Nicolson has provided the foundation of our understanding of the structure of cellular membranes [1]. Recently, this model was challenged by the concept of "lipid rafts" supported by findings of detergent insoluble membrane fragments more concentrated in certain lipids like sphingomyelin and cholesterol [2]. Caveolae are small plasma-membrane invaginations of similar lipid composition. The invaginated structure of caveolae seems to be stabilized by the protein caveolin-1 [3]. Caveolae domains are found in most cell types, particularly in terminally differentiated cells such as adipocytes, muscle cells and endothelial cells [4]. Lipid rafts and caveolae were shown to represent membrane compartments enriched in a large number of signalling molecules whose structural integrity is essential for many signalling pathways [5]. These microdomains seem to be involved in many biological processes mediated by various receptors [6].

Cyclodextrins (CDs), due to their affinity with hydrophobic molecules, are used to modulate membrane cholesterol levels [7, 8]. For this purpose some CDs have been widely used namely β-CD or its derivatives (this group will be called β-CDs). Numerous studies have shown that exposing cells to β-CDs results in removal of cellular cholesterol at a rate depending on the concentration, incubation time, temperature and type of cells [9]. It is important to note that their efficiency is also dependent on the derivative used (methyl-β-CD was shown to be more efficient compared to hydroxypropylated derivatives) [7–9] explaining why methyl-β-CD is the most currently used tool in cell biology to study lipid rafts and caveolae [10, 11]. Various methylated derivatives differently substituted are commercially available. In a previous study, we have

shown that the cholesterol extraction capacity was also dependent on the degree of substitution (D.S.) [12]. Indeed, the low substituted derivative Crysmeb® (as well as trimethyl-β-CD) presented lower affinity for cholesterol while derivatives with D.S. around 2 were more effective. This previous study should predict the potentiality of these molecules to induce changes in cellular functions.

The present work will focus on investigating the effect of β-CDs on caveolae and try to evaluate the relation with the cholesterol extraction capacities of different methylated derivatives.

Because there is not enough consistent information to predict the impact of β-CDs on the non-cholesterol lipid components of the membrane [9], preliminary assays will be performed with different derivatives in order to confirm their specificity towards cholesterol.

As they contain high levels of sphingolipids and cholesterol, lipid rafts and caveolae are low density membrane domains [13]. To isolate these microdomains, different approaches are possible. They can be extracted from other cellular material, generally based on their relative insolubility in certain detergent or nondetergent conditions. They are then isolated by virtue of their high buoyancy when centrifuged on a density gradient consisting either of sucrose or Optiprep® [4, 14]. A conventional method to prepare lipid rafts uses Triton® X-100 at low temperature (4 °C) but a variety of other detergents have been proposed including Nonidet® P40, octylglucoside, CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), Lubrol® WX or PX and Brij® 56, 96, or 98 [13, 15]. Several methods have been established to isolate rafts from cells fractionated in the absence of detergent. Among these, Song et al. [16] recovered cells in a pH 11 sodium carbonate buffer. Following sonication of the lysate, the rafts are isolated by centrifugation on a discontinuous 5/35/45% sucrose gradient where they concentrate at the 5 and 35% sucrose interface [13, 15, 16].

This relatively easy detergent-free procedure, associated with caveolin-1 detection in the fractions obtained after ultracentrifugation, will be used here to assess the effect of CD treatment on endothelial cells microdomains. By reducing cholesterol levels, CDs could lead to the disruption of rafts [17] or caveolae, interfere with processes involving these microdomains and have thus biological and potential medical applications [6].

Experimental

Materials

β-cyclodextrin (β-CD) and dimethyl-β-cyclodextrin (Dimeb; D.S. 2.0) were kindly donated by Cyclolab (Budapest, Hungary). Hydroxypropylated β-cyclodextrin (HP-β-CD; D.S. 0.61) and Kleptose® Crysmeb (Crysmeb; D.S. 0.5) were given by Roquette Frères (Lestrem, France). Randomly methylated β-cyclodextrin (Rameb; D.S. 1.8) was offered by Wacker-Chemie GmbH (Munich, Germany). Methyl-β-CD (Mβ-CD; D.S. 1.9) and trimethyl-β-cyclodextrin (Trimeb, from Fluka; D.S. 3.0) were purchased from Sigma-Aldrich (Bornem, Belgium).

Adult bovine aortic endothelial cells (ABAEC) were a kind gift from the Molecular Biology and Genetic Engineering Unit of the University of Liège. Human umbilical vein endothelial cells (HUVEC) were obtained from Lonza (Verviers, Belgium) as well as foetal calf serum (FCS), non-essential amino acids (NEAA), Dulbecco's modified Eagle's medium (DMEM) high glucose with Ultraglutamine and phosphate buffer saline (PBS). MCBD 131 culture medium, L-glutamine and penicillin–streptomycin were from Gibco®-Invitrogen. The endothelial cell growth supplement (ECGS) was purchased from Becton Dickinson Biosciences and basic recombinant mouse fibroblast growth factor (bfGF) from R&D Systems. Gentamicin (Geomycine®) was obtained from Schering-Plough and amphotericin B (Fungizone®) from Bristol-Myers Squibb. Concerning secondary antibodies, polyclonal swine anti-rabbit immunoglobulins/HRP and Alexa Fluor® 488 goat anti-rabbit IgGs were respectively from Dako Cytomation (Carpinteria, USA) and Molecular Probes®. All other chemicals and cell culture reagents were purchased from Sigma-Aldrich (Bornem, Belgium) or Merck (Darmstadt, Germany).

Cell culture

HUVEC were grown on 2‰ pork skin gelatin-coated culture dishes in MCBD 131 medium supplemented with 20% FCS, 2 mM glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, 25 µg/mL gentamicin, 0.5 µg/mL amphotericin B, 50 µg/mL ECGS and 7 U/mL heparin. They were used at passages between 2 and 6.

ABAEC were grown in DMEM high glucose with Ultraglutamine supplemented with 10% FCS, 100 U/mL penicillin, 100 µg/mL streptomycin, 1% NEAA and 1 ng/mL bFGF. They were used at passages not higher than 11.

Cells were maintained in a humidified 5% CO_2 and 95% air atmosphere at 37 °C and supplied with fresh medium every 2 or 3 days.

Cyclodextrin treatments

CD test solutions were prepared by dissolution in serum- and growth supplement-free medium. CD mass was corrected for water content when calculating the molarity

using a Mettler DL 35 Karl-Fischer titrator. The solutions were sterilized by filtration in aseptic conditions.

Each condition was tested on one 10 cm dish of cultured cells grown to near confluence: 10 mL of CD solutions were applied on cell monolayers for a 1 h incubation. The cells were rinsed twice on ice with PBS before further analysis.

Phospholipids measurements

As well as for cholesterol measurements, the Folch's method, using chloroform/methanol at a 2:1 volume ratio [12, 18], gave good results for phospholipids extraction in HUVEC. The cells were scraped in ice-cold PBS, centrifuged at 1,000 rpm and the resulting pellet was homogenized in 500 µL of distilled water by sonication. Phospholipids were extracted with the organic solvent mixture from a volume of cell lysate corresponding to 150 µL (at least in duplicate). After centrifugation at 4,000 rpm, the organic phase was collected and evaporated to dryness. The phospholipids contents were evaluated by an enzymatic method (LabAssay Phospholipid, choline oxidase—DAOS method, Wako Chemicals, Neuss, Germany). Using this colorimetric assay, phospholipids are hydrolyzed to choline in a reaction catalyzed by phospholipase D. The oxidation of choline in betaine by choline oxidase leads to a quantitative production of hydrogen peroxide, which causes DAOS (*N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt) and 4-aminoantipyrine to undergo an oxidative condensation catalyzed by peroxidase, producing a blue pigment. The absorbance of the blue color is thus proportional to phospholipids contents. This assay was successfully used for microplate reading using a Labsystems Multiskan MS microplate reader at 595 nm.

Rafts/caveolae isolation

The hyperosmotic carbonate method, a detergent free method described by Song [16] was used for caveolae isolation with minor modification [4, 14]. The cells were scraped into 2.5 mL of 0.5 M sodium carbonate containing protease inhibitors and transferred to a prechilled Potter homogenizer to be homogenized with 20 strokes on ice. They were then homogenized in a glass tube using the Ultra-Turrax® T25 basic (IKA®-Werke, Staufen, Germany) equipped with a S 25 N—10 G dispersing tool (three 20 s bursts at medium speed). This step was followed by sonication (three 20 s bursts at 50% of maximal power) and an aliquot of the homogenate was taken for protein determination using the Lowry technique according to the manufacturer's instructions (*DC* Protein Assay Kit®, BioRad). In Seton open-top polyallomer centrifuge tubes (14 × 95 mm) (Science Services, Munchen, Germany), 0.5 M sodium carbonate solution was added to determined volumes of lysates in order to obtain similar protein contents in 2 mL. The resulting dilutions were brought to 45% sucrose by the addition of 2 mL of 90% sucrose in MES buffered saline (MBS: 25 mM MES, pH 6.5 and 150 mM NaCl) and overlaid with two layers of 35 and 5% sucrose in MBS containing 0.25 M carbonate (4 mL each). The gradient was then centrifuged using a SW 40 Ti rotor in a Beckman L8-70 Ultracentrifuge (38,000 rpm for 16 h).

Gradient fractionation

For analysis of the resulting gradients, fractions were recovered from the top to the bottom of the centrifuge tubes using the Piston Gradient Fractionator of a BioComp Gradient Station (BioComp Instruments, Fredericton, Canada). With this technology, a piston is forced into the tube from above and it seals against its inside wall, forcing the gradient out of the tube layer by layer. The size of the collected fractions is determined by the length of the piston stroke. A low motor velocity was chosen (0.2 mm/s) in order to avoid perturbations of the gradient and fractions were recovered every 10 mm. The collection system was cleaned out between each fraction. Eight fractions of equal volumes and a "bottom" fraction, corresponding to the end of the tube, were taken.

Western blotting

Detection of caveolin-1, a 21–24 kDa marker protein of the caveolae structures, was performed after the gradient separation. Each fraction was diluted in threefold concentrated Laemmli sample buffer, heated and resolved by SDS-PAGE using 15% polyacrylamide gels. The proteins were transferred onto polyvinylidene difluoride (PVDF) membranes by electroblotting. Membranes were blocked by nonfat dry milk (3% in PBS-Tween® 20 buffer) before a 1 h incubation at room temperature with the anti-caveolin-1 antibody dilution (1:5000 in blocking buffer). Immunoreactive bands were revealed after a 1 h incubation with horseradish peroxidase conjugated anti-rabbit antibody and the signals were detected by chemiluminescence using an ECL Western Blotting Analysis System (Thermo Fisher Scientific) and X-ray film exposure. For semi-quantitative analysis of protein levels, densitometry was performed on digital images obtained from western blots using the Bio-Rad Quantity one 4.6 Software. The amount of caveolin-1 in each fraction was expressed in percentages (%) of the sum of the values measured in each fraction taken as 100%.

Immunofluorescence staining

HUVEC cells were seeded on gelatin-coated glass coverslip in a 12-well plate (25,000 cells/well). After overnight incubation, they were fixed in paraformaldehyde 2% in PBS for 20 min and permeabilized with 0.1% Triton® X-100 in PBS for 10 min. The coverslip was then exposed for 1 h to a 1:200 dilution of the anti-caveolin-1 antibody (in 1% bovine serum albumin) and after to the labelled secondary antibody dilution (1:1000 anti-rabbit Alexa Fluor® 488, 1 h). After washing, the coverslip was mounted with Aqua-Poly/Mont (Polysciences) and viewed with a fluorescence microscope using the appropriate filter (Axiovert 25, Zeiss).

Results and discussion

In order to evaluate the potential effects of CDs on the extraction of cell membrane lipids other than cholesterol, the LabAssay Phospholipid kit was used to assess the phospholipids contents in HUVEC cells. This assay allows the simultaneous determination of different components (phosphatidylcholine, sphingomyelin and lysolecithin). Preliminary assays were performed on an increasing number of cells in order to evaluate the efficiency of the phospholipids extraction protocol. A linear response was obtained with the colorimetric assay confirming that phospholipids removal by the solvent mixture can be well characterized by this method (Fig. 1). Moreover, after a second extraction of the residual, the absorbance values measured were not significant (data not shown), allowing to consider that the phospholipids were completely extracted with a single-step extraction. Compared to the previously described protocol for cholesterol measurements [12], relatively high absorbance values were obtained with low cell number, allowing to perform the following analyses on smaller quantity of cell lysates. The different CD concentrations tested here were chosen according to previous results [12]. This previous study allowed us to select the concentrations that did not induce cell loss by detachment after treatment (recovery of 100% total protein). When compared to the control condition (0 mM CD), none of the β-CDs and concentrations used here induced a significant decrease in the phospholipids measurements (Fig. 2). These results seem to be in contradiction with those obtained by phase solubility diagrams as Dimeb and to a lower extend Rameb were able to solubilize phosphatidylcholine [19] but these experiments were performed on purified lipids, a condition far from the complexity of the lipid mixtures in cell membrane. The results of the present study suggest a lack of affinity for phospholipids compared to cholesterol in the tested conditions (i.e. the type of CDs and the very short incubation period used). The inner cavity of β-CD, and as a consequence of its derivatives, should not be as suitable as the smallest CD (α-CD) to interact with phospholipids [20]. Other studies which compared the effect of natural CDs on phospholipids removal from cell [21, 22], found that α-CD was actually the most efficient. A number of studies reported only minimal release of phospholipids from cell membranes under exposure to CDs [9]. For instance, Kilsdonk et al. [8] observed that a maximum of 2% of cellular phospholipids were released from L-cells when using methyl-β-CD but with relatively high concentration and long incubation period (5 mM for 4 h) while no measurable phospholipid was detected in the media with a 10 mM HP-β-CD solution for 2 h. Niu et al. [23] noted that the amount of phospholipids extracted from rod outer segment disk membranes became significant at concentrations of methyl-β-CD above 15 mM. Differences between the protocols used could also explain the better results obtained in some cases. As an example, β-CD was able to extract up to about 25% phospholipids from erythrocytes [21] but the study was performed in suspension allowing better interactions contrary to experiments performed on

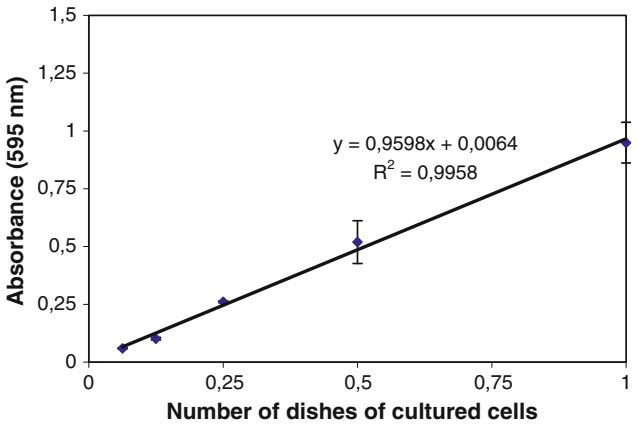

Fig. 1 Phospholipid assay after extraction by the Folch's method on increasing HUVEC cell number ($n = 2$)

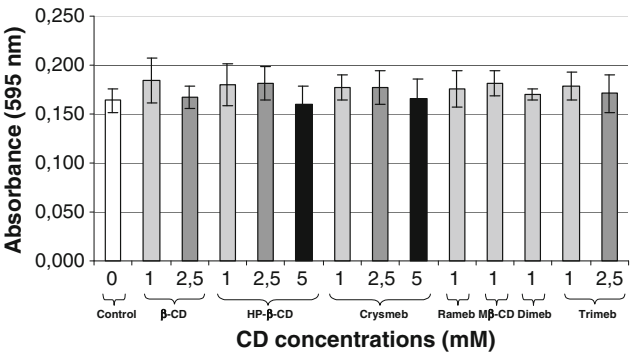

Fig. 2 Phospholipids determination in HUVEC cells after treatment with different CDs and concentrations ($n \geq 9$)

cells in monolayer. In papers reporting high efficiency [22, 24], phospholipids measurements were done in the CD solutions incubated with cells, a procedure which differs from the present method that evaluated the cellular phospholipids contents. By determining lipids released in the incubation buffer, no difference can be done between the amounts extracted by CDs or due to cell detachment and/or lysis. Particular attention is essential when comparing results as some authors consciously used toxic concentrations to study the release of phospholipids from cells [22]. Finally, results obtained on enterocytes [25] are consistent with ours as methyl-β-CD removed the major part of microvillar cholesterol without affecting the membrane contents of phospholipids. Despite the contradictions, all the authors are in accordance with the potency of β-CDs to preferentially extract cholesterol from cell membranes. Their high specificity towards cholesterol should explain their potential implication in lipid raft disruption as it has extensively been described in the literature for methyl-β-CD.

The next step was to try to evaluate the effect of different β-CDs, especially the methylated derivatives, on membrane microdomains in order to assess the impact of the substitution on their disruption.

A detergent-free method using sodium carbonate was chosen to study caveolae which can be detected by their specific marker protein (caveolin-1). According to the literature caveolae should be located, due to their enriched composition in cholesterol, in the light fractions of density gradients [14, 26]. Discontinuous sucrose gradients were obtained by overlaying three solutions of different concentrations (45, 35 and 5% m/V sucrose) in centrifuge tubes. The densities of the three stock solutions were evaluated experimentally by weighting known volumes and calculations were performed in order to obtain values in milligram per millilitre. The densities of nine fractions (noted F1 to F9 from top to bottom) prepared without sample and recovered after ultracentrifugation as described above were also evaluated and compared to fractions obtained before ultracentrifugation (Fig. 3). The figure shows that the ultracentrifugation step does not induce any perturbation of the gradient which remains discontinuous. Caveolae should be located at the interface between 5 and 35% sucrose as stated before. According to the density measurements illustrated in Fig. 3, we were able to determine the localization of this interface (when using our fractionation protocol) in the fraction F3. Moreover, by comparison to the value obtained for the 35% sucrose stock solution, it can be stated that F4 belongs to the heavier fractions.

The first experiment was performed using HUVEC cells treated or not with the methylated β-derivative generally used in cell biology experiments (Fig. 4). Mβ-CD was used

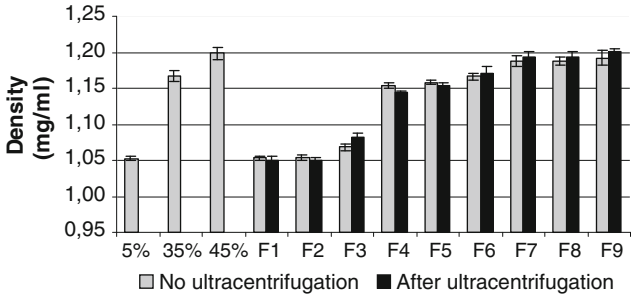

Fig. 3 Densities of sucrose stock solutions (*left part*) and of their resulting fractions (before and after ultracentrifugation) (*right part*)

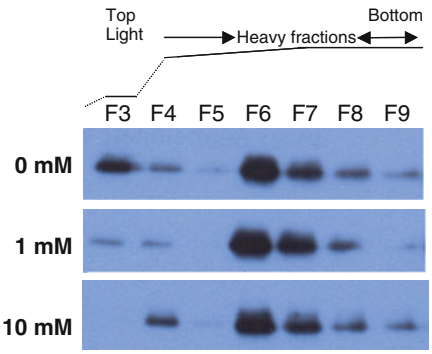

Fig. 4 Effects on caveolae for HUVEC cells treated with Mβ-CD solutions (0, 1 and 10 mM)

at 0, 1 or 10 mM. No caveolin was detected in the top of the tubes (fractions F1 and F2, not shown) confirming the localization of caveolae at the interface between lighter and heavier fractions. Even in control conditions (0 mM CD), caveolin is not only present in the light fractions but also found in the heavier ones suggesting a probable intracellular localization of the protein. To confirm this hypothesis, immunofluorescence staining was performed on untreated HUVEC cells using the Alexa Fluor® 488 conjugated secondary antibody to detect the anti-caveolin-1 antibody (Fig. 5). As shown on the figure, caveolin-1 is both membrane-associated and localized inside the cells [5]. It has indeed been reported to be present in multiple subcellular locations [27]: the presence of non-caveolar pools of caveolin in recycling endosomes, the trans-Golgi network and in mobile chaperone complexes is now recognized [28]. Structures containing caveolin-1 are found in endothelial cells including detached plasmalemmal vesicles and tubular-vesicular channels [29]. Despite these considerations, the first assay showed a little displacement from F3 to the heavier fractions when using Mβ-CD at 1 mM, the effect being slightly more pronounced with 10 mM (Fig. 4). This concentration, generally used in the literature to study lipid rafts, was thus chosen for the further trials.

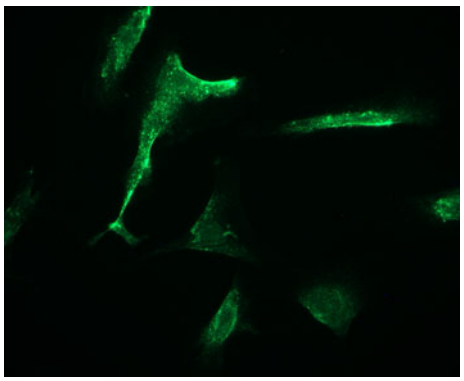

Fig. 5 Immunofluorescence staining of caveolin-1 in HUVEC cells (40× magnification)

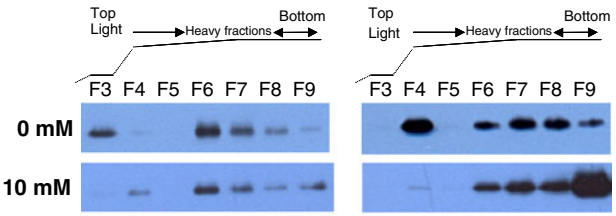

Fig. 6 Effects on caveolae for HUVEC (*left part*) versus ABAEC cells (*right part*) treated with Dimeb solutions (0 and 10 mM)

In a second experiment, Dimeb, another CD exhibiting also good cholesterol extraction capacity was used in order to evaluate its potential stronger effect on another cell line. As a matter of fact, Labrecque et al. [4] reported good results on caveolae disruption when using bovine aortic endothelial cells (BAEC) and the hyperosmotic carbonate method. The Fig. 6 illustrates the results obtained on HUVEC and ABAEC cells. A displacement to the heavier fractions was observed, the stronger effect being obtained with ABAEC. Indeed, 10 mM Dimeb solution induced an accumulation of caveolin-1 in the bottom of the tube for ABAEC cells. This stronger effect obtained using bovine cells prompted us to use the ABAEC cell line for the following experiments.

The different methylated β-CDs were thus tested on ABAEC cells at 10 mM. The results of three independent experiments (A, B or C) performed on this cell line are presented in Fig. 7. In absence of CD, a significant amount of caveolin-1 was detected in the light fraction F3 corresponding to caveolae localization (Table 1). If we consider each series independently by comparison with the control condition, the CDs induced in each case a displacement from the low (F3) to higher density fractions which is characteristic of caveolae disruption (Fig. 7). The drastic effect of Dimeb already observed in Fig. 6, was confirmed in the experiment A. Although less remarkable, a displacement from the light fraction to the heavier ones was also shown for β-CD, Mβ-CD and Rameb, the two latter ones giving very similar signal patterns (Fig. 7) as confirmed by the semi-quantification of the immunoreactive protein bands (Table 1). This finding indicates that the

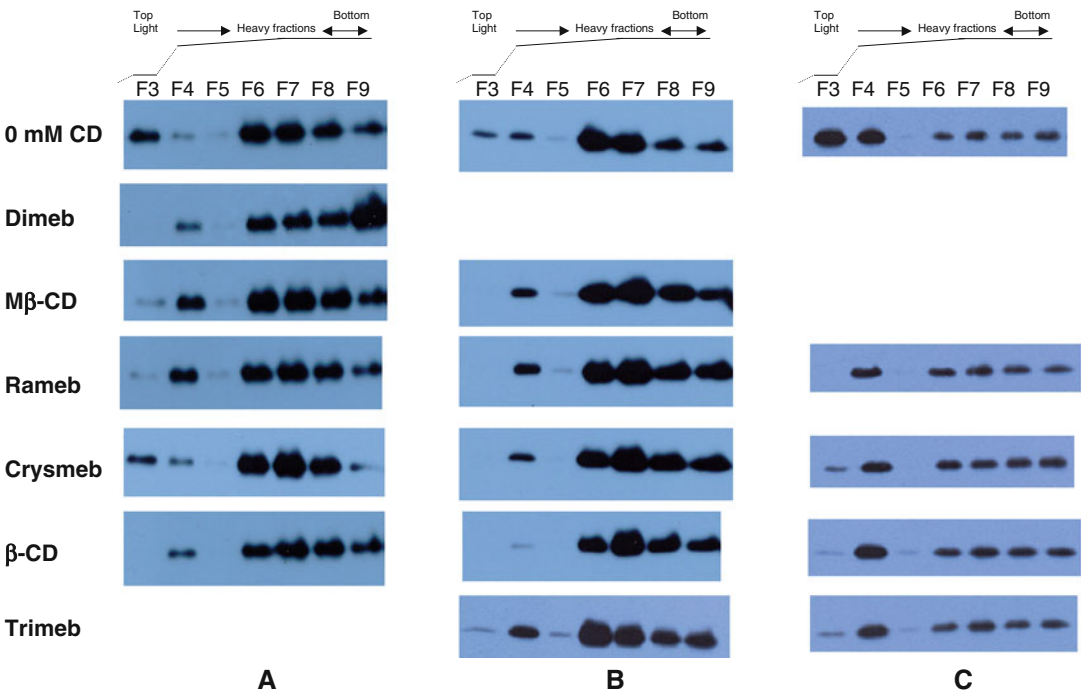

Fig. 7 Effects on caveolae for three independent experiments on ABAEC cells (**A**, **B** and **C**): comparison of treatments with a control solution (0 mM) versus 10 mM CD solutions

Table 1 Distribution (in % of total) of caveolin-1 in fractions F3 to F9 in ABAEC cells ($n \geq 2$)

CD	F3	F4	F5	F6	F7	F8	F9
Control (0 mM CD)	**18.9** ± 13.4	**12.3** ± 11.7	**0.3** ± 0.5	**21.9** ± 12.3	**20.2** ± 7.6	**14.8** ± 5.7	**11.6** ± 2.0
Dimeb (D.S. 2)	**0.0** ± 0.0	**4.6** ± 4.0	**0.0** ± 0.0	**19.1** ± 3.6	**22.2** ± 4.8	**16.7** ± 4.2	**37.4** ± 1.3
Mβ-CD (D.S. 1.9)	**1.1** ± 1.6	**14.1** ± 3.6	**0.0** ± 0.0	**26.6** ± 0.9	**28.1** ± 4.7	**22.5** ± 0.7	**7.5** ± 0.8
Rameb (D.S. 1.8)	**0.4** ± 0.7	**19.2** ± 7.0	**0.2** ± 0.3	**21.5** ± 2.6	**24.6** ± 2.7	**19.7** ± 2.3	**14.3** ± 6.2
Crysmeb (D.S. 0.5)	**4.1** ± 3.2	**11.7** ± 11.1	**0.0** ± 0.0	**22.5** ± 6.9	**27.7** ± 9.8	**19.9** ± 3.8	**14.1** ± 10.9
β-CD (D.S. 0)	**0.6** ± 1.0	**14.3** ± 14.7	**0.4** ± 0.8	**18.9** ± 4.3	**27.8** ± 8.3	**22.9** ± 4.8	**15.0** ± 0.6
Trimeb (D.S. 3)	**3.2** ± 2.1	**21.5** ± 10.2	**0.7** ± 1.0	**21.9** ± 10.4	**21.2** ± 0.4	**14.4** ± 0.6	**17.2** ± 2.7

methyl-β-CD supplied by Sigma is a randomly methylated β-CD and that Rameb or Mβ-CD could be used as well to obtain similar results. The CDs having lower cholesterol extraction capacities [12], namely Crysmeb and Trimeb, seem to have weaker effects on caveolae disruption as shown in Fig. 7. When comparing to the other CDs, a significant amount of caveolin-1 could be detected in the light fraction F3 (Table 1). The semi-quantitative analysis of caveolin-1 signals in fractions F3 to F9 for the three experiments performed on ABAEC cells is shown in Table 1. The results are expressed in each fraction as the percentage of the total amount of caveolin-1 in the sample. These quantifications carried out on the blots from Figs. 6 and 7 for Dimeb confirm the strong effect of this CD for which no caveolin-1 was detected in F3 but high amount was actually displaced to the last fraction F9. Mβ-CD, Rameb and β-CD, as shown in Fig. 7 and Table 1, also induced a displacement of caveolin-1 from F3 to heavier fractions although much less marked than Dimeb. Trimeb and Crysmeb did not induce as strong effects as the other methylated CDs since some caveolin was still present in F3 (Table 1) while the percentages for Mβ-CD, Rameb, β-CD or Dimeb treatments were close or equal to 0%. The results of the present study on microdomains seem in good correlation with the cell cholesterol extraction capacities of CDs that we previously determined [12]. As a matter of fact, the methylated CDs which induced weak cell cholesterol depletions, namely Crysmeb and Trimeb, did not induce such high caveolae disruption at 10 mM.

Conclusion

Preliminary phospholipids assays performed on HUVEC cells showed that β-CDs display a specificity towards cholesterol in our tested conditions as no significant decrease in the cellular phospholipids measurements could be observed.

Concerning their caveolae disruptive capacity, first trials allowed the authors to select the most appropriate CD concentration and endothelial cell line to compare the effect of β-CDs (mainly methylated derivatives). Using methylated CDs a displacement to the heavier fractions of sucrose gradients was observed, which is characteristic of caveolae disruption, the stronger effect being obtained with Dimeb. Crysmeb and Trimeb seemed to have weaker effects which is in agreement with their low cholesterol extraction abilities.

This work enabled to predict the potentiality of β-CDs to induce changes in raft-related cellular functions and to select the better derivatives for future studies. A perspective should be to investigate their effect on proteins co-localized with caveolin-1 in endothelial caveolae i.e. a receptor for the vascular endothelial growth factor (VEGFR-2) implicated in angiogenesis. CDs could thus represent a new potential therapeutic strategy for inhibiting angiogenesis.

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