ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 322 (1999) 247–255

CARBOHYDRATE RESEARCH

Low-molecular-weight dextran derivatives (*f*-CMDB) enter the nucleus and are better cell-growth inhibitors compared with parent CMDB polymers

Patrick Bittoun [a], Thierry Avramoglou [b], Jany Vassy [c], Michel Crépin [d], Frédéric Chaubet [b], Serge Fermandjian [a,*]

[a] *Département de Biologie Structurale, UMR 8532 CNRS, Institut Gustave-Roussy, F-94805 Villejuif, France*
[b] *Laboratoire de Recherches sur les Macromolécules, UMR 7540 CNRS, Université de Paris-Nord, F-93430 Villetaneuse, France*
[c] *Laboratoire d'Analyse d'Images en Pathologie Cellulaire, Université de Paris 7, Institut d'Hématologie, Hôpital Saint Louis, F-75475 Paris, France*
[d] *Laboratoire de Recherches en Oncologie Moléculaire Humaine, UFR Léonard de Vinci, Université de Paris-Nord, F-93017 Bobigny, France*

Received 28 January 1999; revised 12 July 1999; accepted 27 July 1999

Abstract

Carboxymethyldextrans–benzylamide (CMDB) are dextran derivatives that are statistically substituted with carboxymethyl and benzylamide groups. These molecules display a variety of biological effects, one of which is their inhibitory activity against mammary tumor cell growth, both in vitro and in vivo. We and others have previously shown that the effects of CMDB on cell growth are related to their ability to interact with the growth factor FGF-2. The binding modifies the conformation of FGF-2, leading to the suppression of its mitogenic activity. Here, the method previously reported to fragment natural polysaccharide fucans has been applied to CMDB (80,000 g/mol). *f*-CMDB (fragmented CMDB) of molecular weights from 6000 to 20,000 g/mol were found to be more potent inhibitors of MCF7 mammary tumor cell growth than high-molecular-weight CMDB. Confocal microscopy experiments using CMDB and *f*-CMDB labeled with the fluorophore DTAF (5-([4,6-dichlorotriazine-2-yl]amino) fluorescein) indicate that only low-molecular-weight *f*-CMDB penetrate into the nucleus of MCF7 cells. It is thus assumed that the better inhibitory properties demonstrated by *f*-CMDB, compared with CMDB, are related to their better ability to penetrate the nucleus and interact with nuclear targets, including topoisomerase II. The DNA relaxation properties of the latter are inhibited in vitro by both CMDB and *f*-CMDB. These findings could help us to develop models of low-molecular-weight oligosaccharide derivatives exhibiting better antiproliferative and antitumor properties.

Keywords: Dextran derivatives; Radical process degradation; Cell-growth inhibition; Confocal microscopy

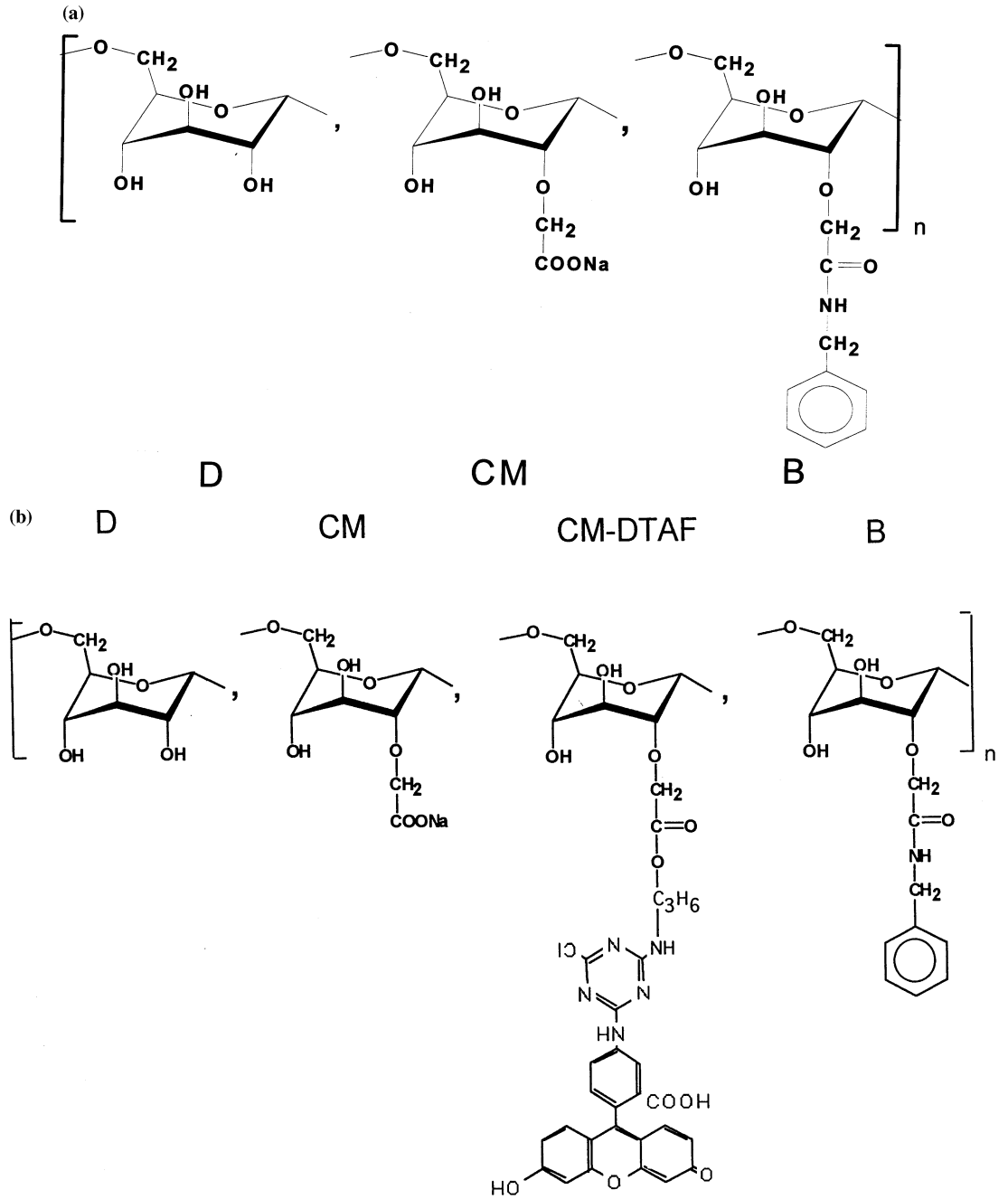
1. Introduction

Carboxymethyldextrans–benzylamide (CMDB) are statistically carboxymethylated and benzylaminated dextrans (Scheme 1) that dis-

Abbreviations: CMDB, carboxymethyldextrans–benzylamide; CMDBS, carboxymethyldextrans–benzylamide sulfonate/sulfate; FGF-2, basic fibroblast growth factor; DTAF, 5-([4,6-dichlorotriazine-2-yl]amino) fluorescein.

* Corresponding author. Tel.: +33-1-4211-4985; fax: +33-1-4211-5276.
E-mail address: sfermand@igr.fr (S. Fermandjian)

0008-6215/99/$ - see front matter
PII: S0008-6215(99)00200-1



Scheme 1. Schematic chemical structures of (a) CMDB (carboxymethyldextrans–benzylamide) derivatives and (b) DTAF (5-([4,6-dichlorotriazine-2-yl]amino) fluorescein)-CMDB.

play some interesting biological activities, such as the inhibition of mammary tumor cell proliferation in vitro and in vivo in animal models [1–4]. It has previously been shown that the sulfonated versions of CMDB, namely CMDBS, possess several heparin properties[1] such as anticoagulant activity [5–7], cell-growth modulation [7,8] and FGF-2 protection [9,10]. Depending on the cell type, the polymer concentration, and the degree of substitution (ds) by carboxymethyl (CM), benzylamide (B) and sulfonate (S) groups, CMDBS modulate cell growth either negatively [11,12] or positively [8,13].

CMDB lack the anticoagulant activity of CMDBS but are more potent inhibitors of cancer cell growth than CMDBS [1,2]. Previous studies carried out on CMDB concluded

[1] For a review see Ref. [5].

that the growth factor FGF-2 is an important target for CMDB [3]. These CMDB probably behave as a class of low-affinity receptors for FGF-2, similarly to heparin and heparin-related compounds [14,15], which help FGF-2 to bind to its high-affinity receptors [16,17]. CMDB have also been shown to interfere with the FGF-2 autocrine loop that controls human breast epithelial HBL100 cell proliferation [3] and to displace FGF-2 from its receptor, but they do not affect the binding of insulin-like growth factor (IGF-1) and epidermal growth factor (EGF) to their receptors. In fact, CMDB, but also their fragments *f*-CMDB (fragmented CMDB), modify the secondary structure of FGF-2 upon their binding to this growth factor [18]. No significant difference has been found between CMDB and *f*-CMDB for their binding to FGF-2 concerning the dissociation constant and the stoichiometry, although these compounds do not exert the same effects on cell-growth inhibition.

The present work was undertaken partly in an attempt to elucidate the origin of the above-reported difference. We studied the effects of CMDB with different molecular weights on cell growth in parallel to their capacity for cell penetration. *f*-CMDB were obtained from CMDB using a method developed for the degradation of natural polysaccharides by Nardella et al. [19]. The inhibitory effect of the compounds was measured on the MCF7 mammary tumor cell growth and was found to be higher for *f*-CMDB than for CMDB. Yet, CMDB and *f*-CMDB exhibit the same in vitro inhibitory effect against topoisomerase II, one potential nuclear target for antitumoral drugs. The difference in the activity of CMDB and *f*-CMDB on cell growth is thus discussed in terms of differential cell penetration and accessibility to the nuclear target topoisomerase II. Cell penetration was assessed by confocal microscopy analysis with DTAF-labeled CMDB and *f*-CMDB derivatives.

2. Experimental

Materials.—Common chemicals for synthesis and fragmentation were purchased in analytical grade from Carlo Erba, Fluka, E. Merck and Sigma, and were used without further purification. Standard Heparin H410 was obtained from the Institut Choay-Sanofi (France).

Synthesis

Synthesis of CMDB. CMDB were prepared from T40 dextran and characterized as previously described [6,7,20]. The final CMDB products were obtained with a ds in CM and B groups of 0.67 and 0.33, respectively.

Preparation of DTAF-labeled CMDB. In order to label CMDB with the DTAF fluorochrome (5-([4,6-dichlorotriazine-2-yl]-amino) fluorescein, Sigma), some amino groups were introduced after the carboxymethylation step (before the benzylamidation), using bromopropylamine (ds 0.025). This allows the fixation of the DTAF fluorophore to the propylamine moiety as previously described [21,18].

Fragmentation of CMDB and labeled CMDB. Polymers were fragmented into low-molecular-weight products according to Nardella et al. [19]. *f*-CMDB denotes fragmented labeled and unlabeled CMDB. Briefly, fractions were obtained from high-molecular-weight CMDB (80,000 g/mol) by using radical process degradation. This degradation proceeds through the formation of free radicals from the hydrogen peroxide–cupric redox system at pH 7.4 [22]. Full-size CMDB (0.03 mmol, 2 g) were dissolved in 200 mL of water. The degradation was conducted by pouring copper acetate monohydrate (20 mL, 0.4 mmol, 60 mg) into this solution. Degradation began once hydrogen peroxide was added with a peristaltic pump (Mettler) at a selected flow rate of 5 mL/h. The temperature was controlled in the reactor and the pH maintained at 7.5 by the automatic addition of 2 M NaOH. Reactions were conducted either at (1) 50 °C with 0.9% H_2O_2 or at (2) 35 °C with 0.09% H_2O_2, varying the reaction time from 20 to 180 min. Low-molecular-weight *f*-CMDB ranging from 6000 to 10,000 g/mol were obtained according to reaction (1) and for fragments ranging from 10,000 to 50,000 g/mol, CMDB were treated according to reaction (2). Samplings were made at 20 min (8000

g/mol), 60 min (6000 g/mol) and 180 min (4000 g/mol) using reaction (1) and at 20 min (23,000–50,000 g/mol), 40 min (15,000 g/mol), 60 min (10,000 g/mol) and 180 min (7000 g/mol) using reaction (2). Reactions were stopped by adding Chelex 100 chelating resin (Biorad) to the slightly acidic medium, thus allowing better elimination of cupric ions. The solution was neutralized with NaOH 0.1 M and desalinated on a Sephadex G15 column (5.2×52 cm, Pharmacia). Samples were collected under double detection UV spectrometry at 254 nm and refractometry. Fractions were then concentrated and recovered by lyophilization. The same protocol was used to obtain the labeled *f*-CMDB. The DTAF-labeled CMDB were depolymerized to produce labeled *f*-CMDB using reaction (1) (6000 g/mol). Controls indicated no loss of fluorescence efficiency.

The chromatographic molecular weights were determined by high-performance steric exclusion chromatography using a Licrospher Si 300 diol column (Merck-Clevenot) and a Hema Sec Bio 40 column (Altech) connected in series to a 510 model pump (E. Merck) and to a Rheodyne injection valve with a 100 μL loop. Columns were calibrated with standard polysaccharides, pullulans of various molecular weights (Polymer laboratories, Interchim, Fluka), dextran, sucrose and glucose (Sigma). Samples were eluted in a NaCl solution 0.15 M, Na_2HPO_4 0.05 M pH 7.3 under refractometric monitoring (Jobin–Yvon, France). Each peak molecular weight was determined using the GPC Chromstar software (Brüker, Merck-Clevenot).

Biological evaluation

Cell culture. MCF7 breast epithelial cell lines were routinely grown in Dulbecco's modified Eagle's (DMEM) (Gibco, NY) complemented with 10% fetal calf serum (FCS) (Gibco, NY), 2 mM L-glutamic acid, 1 mM sodium pyruvate and 50 IU–50 mg/mL penicillin–streptomycin mixture (Gibco). The cell-growth study was conducted as previously described for the HBL100 cell line [2]. Briefly, cells were plated at a density of 2×10^4/well in Falcon 24-well tissue culture plates (Polylabo, France) for 24 h in 10% FCS–DMEM to allow cell adhesion. Cells were then washed with DMEM and incubated for 3 days with the CMDB (15 μM) in 2% FCS–DMEM, 0.1% BSA medium. Cells were then washed with PBS, dissociated with 0.025% trypsin–EDTA (Gibco, France) and counted using a Coulter-counter (Coultronics, France).

Cell preparation. Cells were plated on coverslips at a density of 2×10^4/well in Falcon 6-well tissue culture plates (Polylabo, France) for 24 h in 10% FCS–DMEM. Coverslips were washed with DMEM and incubated for 15 min or 1 h with labeled CMDB and *f*-CMDB at 10, 1 or 0.1 mg/mL at room temperature. Coverslips were then rinsed three times with PBS, blocked with 0.2% BSA–PBS and fixed for 15 min in 4% paraformaldehyde. After rinsing with 0.2% BSA–PBS and water, coverslips were dried, inverted and mounted in 80% glycerol, 20% PBS and 0.1 M *N*-propylgallate (Sigma).

Fluorescence confocal microscopy. Digitized images of 2 μm confocal optical sections of preparations were acquired in horizontal sections (*z*-scan) using a Biorad MRC 600 laser scanning microscope. A multiple line argon laser beam (25 mW) was used with the COSMOS software package (Biorad) mounted on a Nikon plan apochromat immersion objective ($\times 60$) with a high numerical aperture. Labeled CMDB were excited under a 488 nm laser line and optical sections collected with an LP emission filter at 515 nm. Serial *z*-sections at precise positions in the nucleus were obtained by averaging and collecting a constant number of accumulations and steps of 0.5–1 μm were carried out between each focal plane in a horizontal section. Sections therefore correspond to intranuclear fluorescence. No fluorescence was observed with free DTAF.

Enzyme assay.—Yeast DNA topoisomerase was obtained from overexpression in *Saccharomyces cerevisiae* as previously described [23]. The enzyme DNA substrate supercoiled pBR322 plasmid was purchased from Sigma and was used without further purification. The relaxation of the supercoiled plasmid DNA was conducted as reported earlier [24]. Briefly, reactions were performed in standard buffer (10 mM Tris–HCl, pH 7.4, 150 mM KCl, 5 mM $MgCl_2$) containing 0.5 mM ATP and 150

Table 1
Protocols used for degradation of dextran derivatives into low-molecular-weight fragments [a]

Compound Mc (g/mol)	Time (min)	Temperature (°C)	$[H_2O_2]$ (%)	ds CM	ds B
3500	180	50	9	0.65	0.35
4000	180	50	9	0.66	0.34
6000	180	50	9	0.69	0.31
7000	180	35	0.9	0.61	0.39
10,000	120	35	0.9	0.65	0.35
15,000	90	35	0.9	0.65	0.35
25,000	90	35	0.9	0.65	0.35
50,000	20	35	0.9	0.59	0.32

[a] This table details the composition of the CMDB tested in this study and summarizes the results of the degradation conditions; ds with CM and B is calculated from elemental analysis of nitrogen and from titration of the carboxylic groups. Mc, chromatographic molecular weight (SD is $\pm$ 500 g/mol).

ng of pBR322 DNA. Reaction was initiated at 30 °C for 10 min by addition of topoisomerase II and terminated by addition of 1% SDS, 0.05% bromophenol blue and 10% sucrose, final concentrations. Samples were submitted to electrophoresis in 1% agarose gels at 2 V/cm for 18 h in Tris–borate–EDTA buffer at pH 8. Photographic negatives of ethidium bromide-stained agarose gels were scanned with a Joyce-Loebl Chromoscan 3 densitometer and the peak areas of supercoiled DNA determined.

3. Results

The starting CMDB molecules (80,000 g/mol) were obtained with an average ds in CM and B groups of 0.67 and 0.33, respectively (Scheme 1(a)). In order to label CMDB with the DTAF fluorophore, some primary amino groups were introduced during the carboxymethylation process with a final ds of 0.03 (Scheme 1(b)). CMDB and their fragments possess the same overall composition in CM and B groups and have molecular weights ranging from 3500 to 50,000 g/mol. Sample compositions are presented in Table 1, together with the results of the various degradation experiments. Results show that CMDB fragments of desired molecular weights can be obtained by combining heating, reaction duration and amount of H_2O_2. Note for instance that a temperature of 50 °C and hydrogen peroxide at 9% favor the production of low-molecular-weight CMDB. Labeled *f*-CMDB (6000 g/mol) were obtained without loss of fluorescence efficiency from fragmentation of DTAF-labeled CMDB.

At the end, we disposed of high- and low-molecular-weight labeled or not labeled CMDB (CMDB and DTAF-CMDB, *f*-CMDB and DTAF-*f*-CMDB), allowing a mass effect study of CMDB regarding both cell-growth inhibition and cell internalization.

Fragmented f-*CMDB are more potent inhibitors of cell growth than full-size CMDB.*— In previous work, we and others have shown that modified dextrans inhibited the growth of various breast epithelial cell lines [2,25]. The effect varied according to the cell line examined, the CMDB concentration and the degree of CMDB substitution by CM and B groups. Now, we report on the CMDB size effects. Fig. 1 shows the effects of CMDB and of their fragments *f*-CMDB on the growth of breast cancer MCF7. Globally, *f*-CMDB exert a

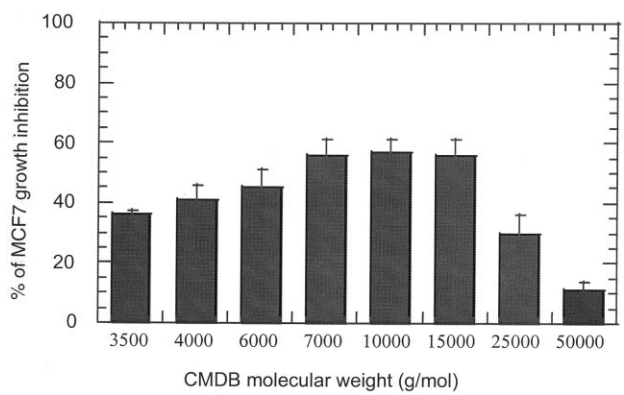


Fig. 1. Histogram reflecting the activities of CMDB with various molecular weights on MCF7 cell proliferation.

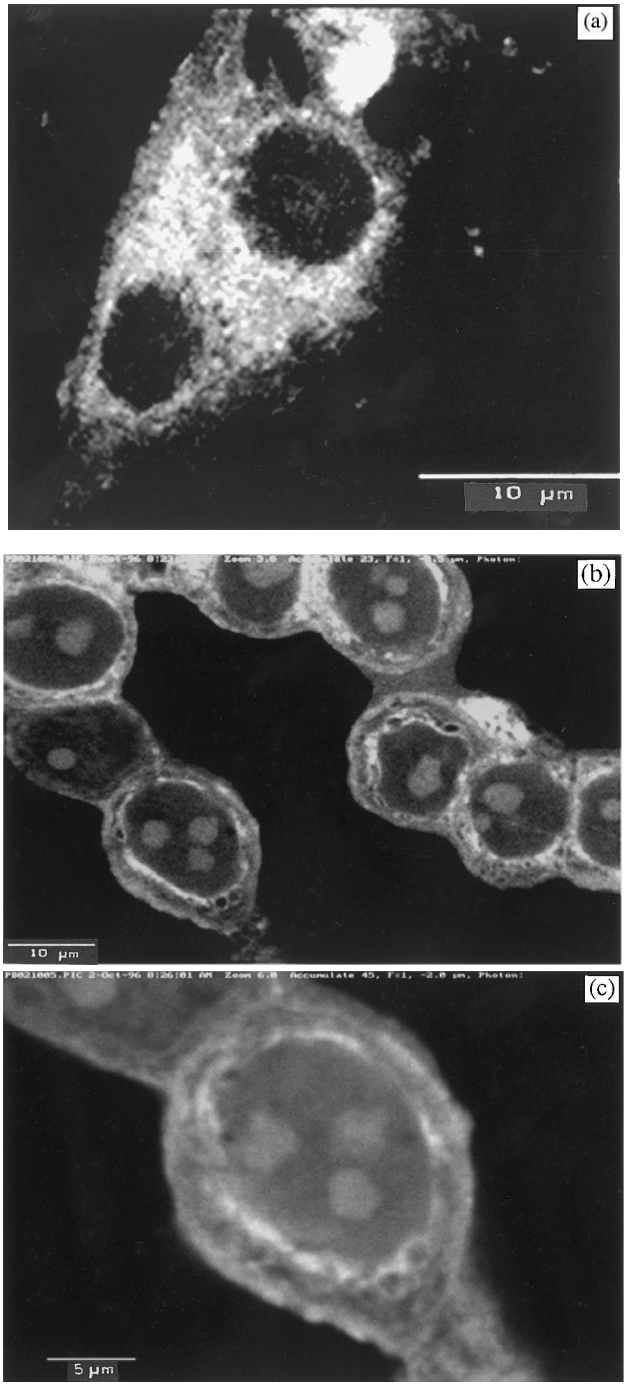


Fig. 2. Confocal microscopic analysis of the fluorescent staining of MCF7 cells with: (a) DTAF-CMDB and (b,c) DTAF-*f*-CMDB. MCF7 cells were incubated for 30 min at 37 °C with 0.1 µg/mL of: (a) high (CMDB, 80,000 g/mol) and (b,c) low (*f*-CMDB, 6000 g/mol) molecular weights. Controls performed with DTAF alone are not shown.

greater inhibitory effect on the growth of these cancer cells than the parent CMDB (80,000 g/mol). Antiproliferative effects are at a maximum for fragments ranging from 6000 to 20,000 g/mol. The same is true for DTAF-labeled CMDB (data not shown).

Detection of low-molecular-weight CMDB in the nucleus by confocal microscopy.—Results of a previous work have suggested that the biological effects of CMDB could be modulated by their ability to enter the nucleus [18]. Here, the propensity of *f*-CMDB (6000 g/mol) and that of their parent CMDB (80,000 g/mol) to penetrate into the cell was assessed by confocal microscopy using the DTAF-labeled derivatives. Such labeling is well adapted to confocal microscopy analysis. The method provides a topological evaluation of CMDB and *f*-CMDB distribution in the cell, and in order to obtain precise staining several exposure times (from 30 min to 2 h) and several labeled CMDB concentrations (0.1 to 10 µg/ml) were assayed. The signal/noise fluorescence allowing the best staining was obtained with a compound concentration of 0.1 µg/mL with 30 min fixation time. Fig. 2(a) shows that weak cytoplasmic fluorescence occurred in the breast cancer MCF7 cells incubated with the high-molecular-weight CMDB. Staining was concentrated in the extracellular matrix, at the cell surface, and in the cytoplasmic area around the nuclear perimeter. No fluorescence was detected in the nucleus, indicating that the nucleolus was not stained. In contrast, abundant low-molecular-weight labeled *f*-CMDB were found around the nucleus and probably also in the nucleolus, as illustrated in Fig. 2(b) and with a greater magnification in Fig. 2(c). In fact, the pattern provided by 2 µm confocal laser sections through the cell revealed that fluorescence was present within the nucleus and highly concentrated around the nuclear envelop. In comparison, cells used as controls incubated with free DTAF did not exhibit a similar pattern of fluorescence (data not shown), indicating that free DTAF does not penetrate into the nucleus.

CMDB inhibit the catalytic activity of DNA topoisomerase II.—The effects of the modified dextrans CMDB on the catalytic activity of topoisomerase II were measured through a relaxation assay of supercoiled pBR322 plasmid. The electrophoresis gel presented in Fig. 3 shows that inhibition of topoisomerase II starts at 100 nM CMDB. CMDB and short fragments, *f*-CMDB, display similar effects on the catalytic activity of topoisomerase II.

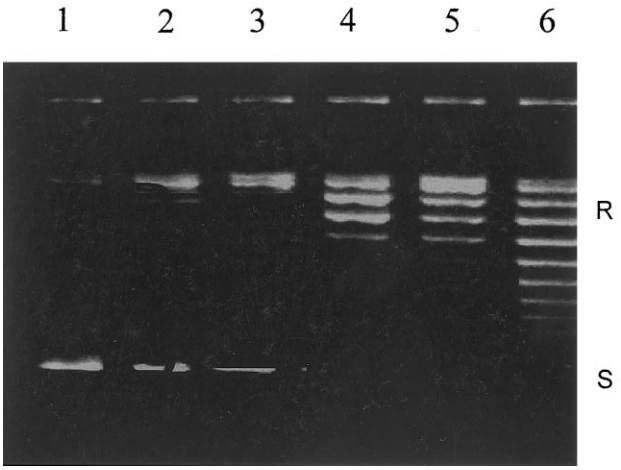


Fig. 3. Inhibition of the catalytic activity of purified yeast topoisomerase II by CMDB as measured by relaxation assay. Supercoiled pBR322 plasmid DNA was relaxed by topoisomerase II in the absence (lane 1) or presence of 500, 100, 10, 5 or 1 nM CMDB (lanes 2–6, respectively). S, supercoiled DNA; R, relaxed DNA.

4. Discussion

One of the aims of this work was to compare the antiproliferative properties of CMDB and *f*-CMDB together with their cellular localization. Radical process degradation of CMDB (80,000 g/mol) yielded low-molecular-weight *f*-CMDB with good reproducibility and in sufficient amounts. This method had previously been applied to the degradation of natural sulfated polysaccharides (fucans: [19], heparin: [26], and dermatan sulfate: [27]). Radical degradation proceeds at neutral pH and is thus more convenient than the classic acidic degradation which precipitates CMDB. Radical degradation followed by gel chromatography separation has also been used successfully for CMDBS (the sulfonated version of CMDB), leading to a molecular-weight distribution ranging from 5000 to 20,000 g/mol. Other authors have observed a molecular-weight dependency of the anticomplementary and anticoagulant activities of these compounds [28].

The antiproliferative properties of the parent CMDB had already been reported [1,2], while nothing was known concerning *f*-CMDB. High-molecular-weight CMDB have been shown to inhibit the cell growth of breast cancer mammary MCF7 by blocking the cells at the G0/G1 transition phase. In comparison, the simpler dextran and CMD remain inactive even at high concentrations. The efficiency of CMDB appears to be associated with their content in both CM and B groups [2], these groups being directly involved in the interactions with important amino acid residues of the growth factor FGF-2 [3,18].

We have used circular dichroism and fluorescence anisotropy measurements to show that the conformation of FGF-2 is significantly altered upon its binding to CMDB and to short CMDB fragments [18]. CMDB and fragments form a stable 1:1 complex with FGF-2, affinities being estimated at 20 ± 10 nM from fluorescence anisotropy analysis. CMDB compete with the FGF-2 receptor for binding to FGF-2 but they do not disturb the binding of IGF-1 and EGF to their receptors. Thus, our previous results have highlighted the binding selectivity of CMDB and their fragments towards FGF-2. However, while CMDB bind rather selectively to FGF-2, heparin competes with CMDB for their binding to FGF-2, strongly suggesting that the CM and B groups of CMDB contribute to the interaction of CMDB with a heparin-binding region of FGF-2. In all cases, it is the resulting change of conformation that is presumed to disturb the binding of FGF-2 to its receptor, and, consecutively, its mitogenic activity.

Present results show that low-molecular-weight CMDB and *f*-CMDB are better inhibitors of MCF7 breast cancer cells than high-molecular-weight CMDB. The best *f*-CMDB exhibit molecular weights ranging from 6000 to 20,000 g/mol (25–100 saccharide units). In contrast, for heparin and sulfated polysaccharide fucans, the higher the molecular weight the greater the antiproliferative activity against smooth muscle cell growth [29,30]. CMDBS, which are polyanionic compounds very similar to heparin, are more potent inhibitors of smooth muscle cell growth compared with their unsulfonated version CMDB [30], but they exert only limited effects on MCF7 cell growth. All these data, which incidentally highlight a cell-type dependency, strongly suggest that sulfated and sulfonated compounds — that is, heparin, fucans and CMDBS — have the same targets. But it

appears likely that the unsulfonated dextran derivatives CMDB have additional properties.

We have previously shown that CMDB and *f*-CMDB fragments exhibit the same binding affinity towards FGF-2 [18]. However, the size of their inhibitory effects on cell growth was different, suggesting that *f*-CMDB and CMDB could either interact with different domains of FGF-2 or that *f*-CMDB have, or accede more easily to, additional targets in the cell. To assess the latter point, we needed CMDB labeled with a fluorochrome. Once obtained, DTAF-labeled CMDB provided DTAF-labeled *f*-CMDB through radical degradation, labeled *f*-CMDB keeping the same inhibitory properties as their unlabeled counterparts.

Confocal microscopy allows us to assess the topological distribution of labeled CMDB and *f*-CMDB molecules in the cell. Abundant labeled CMDB fragments were observed around the nuclear envelope and are also likely to be found in the nucleus and the nucleolus. In contrast, CMDB did not occupy the nucleus but were seen rather as spots of staining in the cytoplasm and in the cell perimeter. In contrast to *f*-CMDB, heparin fragments have been detected only in the nuclear perimeter [31,32]. Yet, this has suggested their implication in the activity of the nucleus, and more particularly, in gene expression, similarly to some heparan sulfates [33]. Moreover, it has been previously suggested that some of the biological effects of FGF-2 could be due to its direct interaction with nuclear effectors [34–38]. In fact, a recent work assumes the presence of functional high-affinity FGF receptors in the nucleus to explain the nuclear biological activities of FGF-2 [39]. Once in the nucleus, the CMDB fragments could also interact with nuclear FGF-2 and/or its receptors and the interaction would be similar to that observed at the cell surface [3,18]. Note, however, that there are many other potential targets for *f*-CMDB in the nucleus. For instance, *f*-CMDB could bind directly to transcription factors and regulate some biological activities at the genetic level, as suggested for heparin [33]. They can also bind to topoisomerase II, which is an enzyme vital for cell life and represents a privileged molecular target for cytotoxic drugs. In vitro studies reveal that CMDB and *f*-CMDB, similarly to heparin and suramin [40], inhibit the catalytic activity of topoisomerase II, likely through direct interactions with basic domains of the enzyme (here and unpublished results). Since in this work it is demonstrated that *f*-CMDB are more potent inhibitors of cell growth than high-molecular-weight CMDB, we may conclude that the difference arises from a better penetration of *f*-CMDB in the nucleus where they can interact with new molecular targets. The better penetration of *f*-CMDB in the nucleus is attested to by a better staining of the nucleolus, where topoisomerase II is known to be present at high concentration [41].

In conclusion, present results regarding the differential effects of CMDB and *f*-CMDB on cell growth suggest that *f*-CMDB can attain and act on nuclear targets that remain inaccessible to larger CMDB. Obviously, additional in vivo experiments are necessary to ascertain the exact nature and number of these molecular targets. This information could be of great interest for obtaining models useful for the development of more efficient compounds derived from CMDB.

Acknowledgements

The authors are grateful to Lorna Saint-Ange for assistance in revising the manuscript.

References

[1] R. Bagheri-Yarmand, J.F. Morere, D. Letourneur, J. Jozefonvicz, L. Israel, M. Crépin, *Anti-Cancer Res.*, 12 (1992) 1646.
[2] R. Bagheri-Yarmand, P. Bittoun, J. Champion, D. Letoumeur, J. Jozefonvicz, S. Fermandjian, M. Crépin, *In vitro Cell. Dev. Biol.*, 30a (1994) 822–824.
[3] R. Bagheri-Yarmand, J.F. Liu, D. Ledoux, J.F. Morère, M. Crépin, *Biochem. Biophys. Res. Commun.*, 239 (1997) 424–428.
[4] R. Bagheri-Yarmand, Y. Kourbali, A.M. Roth, R. Vassy, A. Martin, J. Jozefonvicz, C. Soria, H. Lu, M. Crépin, *Cancer Res.*, 59 (1999) 507–510.
[5] D. Logeart-Avramoglou, J. Jozefonvicz, *J. Biomed. Mater. Res. Appl. Mater.*, 48 (1999) 578–590.
[6] M. Mauzac, J. Jozefonvicz, *Biomaterials*, 5 (1984) 301–304.
[7] F. Chaubet, J. Champion, R. Maïga, S. Maurey, J. Jozefonvicz, *Carbohydr. Polym.*, 28 (1995) 145–152.

[8] D. Letourneur, J. Champion, F. Slaoui, J. Jozefonvicz, *In vitro Cell. Dev. Biol.*, 29a (1993) 67–73.
[9] M. Tardieu, F. Slaoui, J. Jozefonvicz, J. Courty, C. Gamby, D. Barritault, *J. Biomater. Sci. Polymer Edn.*, 1 (1989) 63–70.
[10] M. Tardieu, T. Avramoglou, J. Jozefonvicz, D. Barritault, *J. Cell. Physiol.*, 150 (1992) 194–203.
[11] D. Letourneur, D. Logeart, T. Avramoglou, J. Jozefonvicz, *J. Biomater. Sci. Polymer Edn.*, 4 (1993) 431–434.
[12] T. Avramoglou, J. Jozefonvicz, *J. Biomater. Sci. Polymer Edn.*, 3 (1991) 149–154.
[13] A. Meddahi, F. Blanquaert, J.L. Saffar, M.L. Colombier, J.P. Caruelle, J. Jozefonvicz, D. Barritault, *Pathol. Res. Commun.*, 190 (1994) 923–928.
[14] D. Klagsbrun, *J. Cell. Biochem.*, 47 (1991) 199–200.
[15] M.C. Kiefer, M. Ishihara, S.J. Swiedler, K. Crawford, J.C. Stephans, P.J. Barr, *Ann. NY Acad. Sci.*, 638 (1991) 167–176.
[16] A. Yayon, M. Klagsbrun, J.D. Esko, P. Leder, D.M. Ornitz, *Cell*, 64 (1991) 841–848.
[17] M. Klagsbrun, A.A. Baird, *Cell*, 67 (1991) 229–231.
[18] P. Bittoun, R. Bagheri-Yarmand, F. Chaubet, M. Crépin, J. Jozefonvicz, S. Fermandjian, *Biochem. Pharmacol.*, 57 (1999) 1399–1406.
[19] A. Nardella, F. Chaubet, C. Boisson-Vidal, C. Blondin, P. Durand, J. Jozefonvicz, *Carbohydr. Res.*, 289 (1996) 201–208.
[20] O. Maïga-Revel, F. Chaubet, J. Jozefonvicz, *Carbohydr. Polym.*, 32 (1997) 89–93.
[21] N.A. Stearns, S. Prigent-Richard, D. Letourneur, J.J. Castellot Jr., *Anal. Biochem.*, 247 (1997) 348–356.
[22] F.H. Haber, J. Weiss, *Proc. Roy. Soc. Lond.*, 147 (1934) 332–351.
[23] S.T. Worland, J.C. Wang, *J. Biol. Chem.*, 264 (1989) 4412–4416.
[24] S. Lamhasni, A.-K. Larsen, M. Barray, M. Monnot, E. Delain, S. Fermandjian, *Biochemistry*, 34 (1994) 3632–3639.
[25] J.F. Morère, D. Letourneur, P. Planchon, T. Avramoglou, J. Jozefonvicz, L. Israel, M. Crépin, *Anti-Cancer Drugs*, 3 (1992) 629–634.
[26] N. Volpi, G. Mascellani, P. Bianchini, *Anal. Biochem.*, 200 (1992) 100–107.
[27] N. Volpi, *Carbohydr. Res.*, 255 (1994) 133–144.
[28] B. Crépon, F. Maillet, M.D. Kazatchkine, J. Jozefonvicz, *Biomaterials*, 8 (1986) 248–253.
[29] J.J. Castellot, D.L. Beeler, R.D. Rosenberg, M.J. Karnovsky, *J. Cell. Physiol.*, 120 (1984) 315–320.
[30] D. Logeart, S. Prigent-Richard, C. Boisson-Vidal, F. Chaubet, P. Durand, J. Jozefonvicz, D. Letourneur, *Eur. J. Cell Biol.*, 74 (1997) 385–390.
[31] J.J. Castellot, K. Wong, B. Herman, D.F. Hoover, T.C. Albertini, B.L. Wright, M.J. Caleb, M.J. Karnovsky, *J. Cell. Physiol.*, 124 (1985) 13–20.
[32] T. Bârzu, M. Pascal, M. Maman, C. Roque, F. Lafont, A. Rousselet, *J. Cell. Physiol.*, 167 (1996) 8–21.
[33] S.J. Bush, G.A. Martin, R.L. Barnhart, M. Mano, A.D. Cardin, R.L. Jackson, *J. Cell. Biol.*, 16 (1992) 631–642.
[34] I.J. Mason, *Cell*, 78 (1994) 547–552.
[35] S. Tessler, G. Neufeld, *J. Cell. Physiol.*, 145 (1990) 310–317.
[36] M. Renko, N. Quarto, T. Morimoto, D.B. Rifkin, *J. Cell. Physiol.*, 144 (1990) 108–114.
[37] R.Z. Florkiewicz, A. Baird, A.M. Gonzalez, *Growth Factors*, 4 (1991) 265–275.
[38] A. Joy, J. Moffett, K. Neary, E. Mordechai, E.K. Stachowiak, S. Coons, J. Rankin-Shapiro, R.Z. Florkiewicz, M.W. Stachowiak, *Oncogene*, 14 (1997) 171–183.
[39] E.K. Stachowiack, P.A. Maher, J. Tucholski, E. Mordechai, A. Joy, J. Moffett, S. Coons, M.K. Stachowiak, *Oncogene*, 14 (1997) 2201–2211.
[40] S. Lelievre, Y. Benchokroun, A.K. Larsen, *Mol. Pharmacol.*, 47 (1995) 898–906.
[41] N. Chaly, D.L. Brown, *J. Cell. Biochem.*, 63 (1996) 162–173.