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# Structure–activity studies of heparan mimetic polyanions for anti-prion therapies

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**Abstract**

Polysulfated molecules, as the family of heparan mimetics (HMs) and pentosan polysulfate, are considered among the more promising drugs used in experimental models of prion diseases. Regardless of their therapeutic potential, structure–function studies on these polyanions are still missing. Here, we report the syntheses of a library of HMs of different molecular sizes, containing various sulfation and carboxylation levels, and substituted or not by different hydrophobic cores. The HMs capacities to inhibit the accumulation of PrPres in chronically infected cells (ScGT1-7) and their PrPc binding abilities were examined. Our results showed that an optimal size and sulfation degree are needed for optimum activity, that incorporation of hydrophobic moieties increases compounds efficacy and that the presence of carboxymethyl moieties decreases it. These structural features should be considered on the modelling of polyanionic compounds for optimum anti-prion activities and for advancing in the understanding the mechanisms involved in their biological actions.


*Keywords:* Prion therapy; Heparan mimetic; Rgta; Structure–function; Polyanion; Anti-prion

Transmissible spongiform encephalopathies (TSEs), also called prion diseases, are fatal neurodegenerative disorders which can be developed by most mammalian species. These diseases include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and the Creutzfeld-Jakob disease (CJD) and its iatrogenic variant (vCJD) in human [1]. This last is an infective form of TSEs that can be transmitted via transplants of contaminated organs or tissues or by ingestion of infected biological products [2]. In the past two decades hundreds of iatrogenic prion transmissions have occurred raising concerns about prions transmission from cattle to other species including humans. Currently, there is no effective treatments for TSEs and research of therapeutic agents capable to slow or stop the evolution of these diseases have to keep progressing [3]. The key event in prion disease biology is the conversion and self-propagating refolding of the host-encoded normal cellular protein (PrPc) into the abnormal protease resistant conformation (PrPres) [4]. To date, PrPres is the only specific marker of the infection and the inhibition of its accumulation is often used to evaluate the efficacy of therapeutic drugs. Molecules that directly or indirectly interact with PrPc and/or PrPres, and that

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are able to inhibit the accumulation of PrPres, are currently being proposed and used as potential drug candidates [5]. Sulfated polysaccharides including some members of the family of heparan mimetics (HM) and pentosan polysulfate (PPS) (Fig. 1A and B) are placed among the most active anti-prion drugs. These and some other polyanions have focused a large interest certainly because of the rationale of their therapeutic use. Indeed, heparan sulfate (HS) (Fig. 1C), abundantly found on the amyloid plaques in TSEs [6], was reported as an essential part of the cellular receptor used for prion uptake and as a crucial factor for cell infection [7]. It is well known that interactions of particular proteins with HS are based in the HS sulfation degrees and patterns [8]. Preliminary studies with the HM family of compounds have suggested that their anti-prion activities are associated to the presence of sulfate moieties but information concerning optimal contents or about other structural features is still missing [9]. Documenting on these areas is certainly an important concern on the development of more efficient drugs. Here, we report the chemical synthesis of a library of HM containing various sulfation levels, of different molecular sizes, and substituted or not by different hydrophobic cores. The synthesized HMs capacities to bind to PrPrec and to inhibit the accumulation of PrPres in chronically infected cells (ScGT1-7) [10] were examined. The importance of these structural features for optimal polyanions interactions with PrPc and PrPres are analysed and discussed.

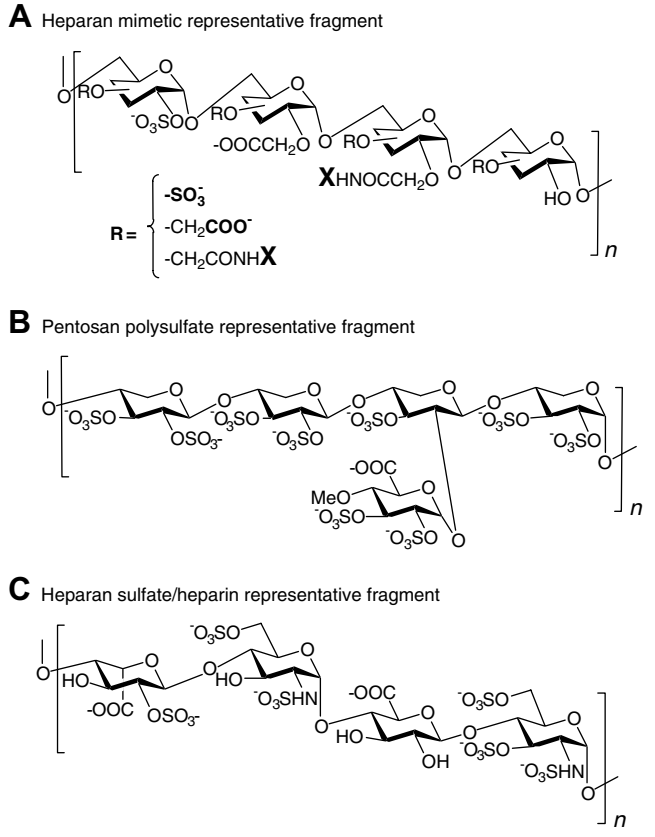


Fig. 1. General formula of representative fragments of sulfated polysaccharides. The HS fragment is here represented by a highly sulfated sequence.

## Materials and methods

*Products*. Dextrans T10 (Mr ≈ 10,000, about 60 anhydroglucoses/chain), T40 (Mr ≈ 40,000, about 250 anhydroglucoses/chain), and T500 (Mr ≈ 500,000, about 3000 anhydroglucoses/chain), were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). 2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EDDQ), sulfur trioxide dimethylformamide ($SO_3$-DMF), and all inorganic salts were from Fluka. Heparin, chondroitin sulfates A, B and C, pentosan polysulfate (PPS), congo red, glucose-6-sulfate (Glc-6S), and all other organic chemicals were from Sigma–Aldrich (Saint Quentin Fallavier, France). Recombinant bovine PrP (PrPrec), which possess the N-terminal fragment, was kindly provided by M.R. Jackman (Veterinary Laboratories Agency, Waybridge, UK). The anti-prion 12F10 and SAF83 antibodies were kindly supplied by Dr. J. Grassi (CEA, Saclay, France). BCA protein assay kit and TMB substrate kit were from Pierce (USA).

*HM syntheses*. General HM synthetic protocols were described elsewhere [11]. Briefly methods were as follows:

*Carboxymethylated dextran $HM_{250}C_{0.5}$*: NaOH 16 M (63 mL at 4 °C) was poured into 100 mL of an aqueous dextran T40 solution (123.5 mmol of anhydroglucose). The reaction mixture was stirred at room temperature 20 min then cooled to 4 °C. Monochloroacetic acid (40.5 g, 430.5 mmol) was added and the reaction was stirred at 50 °C for 50 min before neutralization. The final product was purified by tangential ultrafiltration as stated below. The $HM_{250}C_{0.5}$ sodium salt was obtained at 85% yield (21.2 g, 105.0 mmol, dsCM = 0.5). For preparation of $HM_{250}C_{0.8}$ (dsCM = 0.8) the procedure was repeated on $HM_{250}C_{0.5}$.

*Amidation reaction. Synthesis of $HM_{250}C_{0.5}X_{0.3}$*. To a $HM_{250}C_{0.8}$: (5.0 g, 22.1 mmol of carboxymethylated anhydroglucose) solution in 200 mL acetone/water (1:2, pH 5) at 40 °C were added 5.5 g of 2-ethoxy-1-ethoxycarbonyl-1,2-di-hydroquinoline (22.1 mmol in 20 mL of acetone). After stirring 20 min at 40 °C, 4.8 g of L-phenylalanine methyl ester hydrochloride (22.1 mmol), or equivalent amount of other amine, was added and the pH was adjusted to 6.5. The reaction was stirred at 40 °C for 20 h. The final product was purified by tangential ultrafiltration to give the $HM_{250}C_{0.5}X_{0.3}$ sodium salt at 90% yield (5.3 g, 19.7 mmol of substituted anhydroglucose).

*Sulfation reaction. Synthesis of $HM_{250}C_{0.5}S_{1.0}$*: An aqueous solution of $HM_{250}C_{0.5}$ (5.0 g, 24.8 mmol of substituted anhydroglucose) was eluted through an acidified sulfonic resin (Amberlite IR120). After freeze drying, the product was dissolved in 360 mL of formamide/DMF/2-methyl-2-butene (1:4:1) and 7.4 g of $SO_3$-DMF (48.6 mmol) was added. The reaction mixture was stirred at 30 °C for 2 h. The reaction was quenched with a saturated $NaHCO_3$ solution. The product was purified by tangential ultrafiltration to give the $HM_{250}C_{0.5}S_{1.0}$ at 91% yield (6.9 g, 22.6 mmol of substituted anhydroglucose). Differently sulfated products were obtained by modifying the amount of $SO_3$-DMF.

*Product purification and structure characterization*. Ultrafiltration was performed on a regenerated cellulose membrane (NMWCO, cutoff 1000, Pellicon2, Millipore, MA) against NaCl 1 M and then water. Structure characterization was performed as reported elsewhere [11]. Briefly, degrees of substitution (ds), defined as the number of substituted carboxymethyl (dsCM), carboxymethyl amide (dsX), and sulfate (dsS) groups by glucosidic unit, were determined by NaOH titration (Titroprocessor Metrohm 682) of protonated samples. dsS and dsX were confirmed by elemental analysis of sulfur and nitrogen contents. Absolute molecular weight determinations and size distributions were performed by size exclusion chromatography (SEC) using a TSK Gel G3000-PWXL column (TosoHaas, Cambridge, UK) coupled to a multiangle laser lightscattering photometer (MALLS; Wyatt Technology, CA) connected to a refractive index detector (ERC-7515A, Erma Cr. Inc., France) and eluted by 0.1 M $LiNO_3$. The number of anhydroglucoses by chain (gly/chain) were determined by dividing the polyanion average molecular mass, determined by HPLC, by the average molecular mass of its representative substituted

anhydroglucose unit. To resume, the structural features of compounds used in this study are detailed in Tables 1 and 2.

*Synthesis of heparin-BSA conjugate*. Heparin was covalently bound to BSA in a potassium phosphate buffer (0.2 M, pH 8) as described by Najjam [12]. The resulting product was analysed by the BCA protein assay kit and by the Barbosa GAG assay [13]. The dialyzed solution was used for coating ELISA plates.

*Heparin/HM competition assay towards PrPrec*. ELISA plates were coated with the heparin-BSA complex (5 μg/mL equivalent BSA; 100 μl/well). The plate was incubated 1 h at room temperature, washed with PBS-Tween 20 0.05%, blocked with PBS-BSA 3% and rewashed. Polyanions solutions at various concentrations (0, 0.1, 1, 10, 100, 1000, 10,000, and 100,000 ng/mL in PBS-BSA 1%; 50 μL/well in triplicate) were added followed by addition of a PrPrec solution (200 ng/mL in PBS-BSA 1%; 50 μL/well). Plates were incubated 1 h at room temperature, washed, and the 12F10 antibody (1:1000 in PBS-BSA 1%; 100 μL/well) was added. An anti-rabbit peroxidase antibody (0.3 ng/mL in PBS-BSA 1%) was used to reveal PrPrec using the TMB substrate kit.

*Cell culture*. ScGT1-7 cells (GT1 hypothalamic neuronal cells chronically infected with the Chandler scrapie strain), kindly provided by S. Lehman, (Montpellier, France), were cultured and maintained as described previously [10], except that DEMEM was replaced by Opti-MEM (Invitrogen, France) supplemented with 5% fetal calf serum (FCS), 5% fetal horse serum, 1% penicillin–streptomycin, and 1% sodium pyruvate.

*Drugs treatment of cells*. 96-well plastic plates were used to seed 2000 cells per well. Cell medium solutions of the polyanions at various concentrations (0, 0.01, 0.1, 1, 10, 100, 1000, and 10,000 ng/mL in NaCl 0.9%) were added in duplicate to the corresponding wells and the plates were incubated for 4 days at 37 °C in 5% of $CO_2$.

*Analysis of PrPres levels*. A Dot-blot assay was used as previously described [14]. Briefly ScGT1 cells in a 96-well plates were rinsed with PBS and lysed at 4 °C in a 50 mM Tris/HCl, pH 7.4 containing 0.5% sodium deoxycholate and 0.5% Triton X-100. Wells were treated with proteinase K (5 μg/ml final concentration) for 30 min at 37 °C. Pefabloc was added at 0.8 mM final concentration before applying wells contents to a nitrocellulose membrane by using a dot-blot system. The membrane was treated with 3 M guanidine thiocyanate and blocked with 5% of skim milk in PBS/Tween 20 0.1%. PrPres was detected by incubating the membranes with the SAF83 antibody for 1 h at room temperature, followed by incubation with an alkaline phosphatase-conjugated antimouse antibody. Immunoreactivity was detected by an enhanced chemiluminescence probe (ECL kit, Amersham) from images taken through a light box illumination with a

Table 1
Some known polyanions and their structural features including their molecular length represented by the average number of glycosidic units in the chain, their degrees of sulfate substitution (dsS), carboxylate substitution (dsC), and hydrophobic group substitution (dsX)

| Polyanion | Structural features | | | | Binding $EC_{50}$ (μg/mL) |
|---|---|---|---|---|---|
| | *n* gly[a] | dsS | dsC | dsX | |
| PPS | ~25 | 2.0 | 0.1 | (OMe) 0.10 | 80 |
| CS-A | ~50 | 0.5 | 0.5 | (NAc) 0.50 | >100 |
| CS-B | ~50 | 1.0 | 0.5 | (NAc) 0.50 | >100 |
| CS-C | ~50 | 0.5 | 0.5 | (NAc) 0.50 | >100 |
| HS | ~50 | 0.3 | 0.5 | (NAc) variable | >100 |
| Heparin | ~50 | 1.1 | 0.5 | (NAc) <0.2 | >100 |
| HM5004 | ~250 | 1.3 | 0.3 | — | 5.0 |
| HM2602 | ~250 | 0.7 | 0.9 | (BnNH) 0.2 | 0.9 |

$EC_{50}$ represents the concentration needed to inhibit 50% of PrPrec binding to immobilized heparin.
[a] Number of glycosidic units.

Table 2
Synthesized HMs and their structural features including: molecular length (*n* gly/cain), degrees of sulfate substitution (dsS), of carboxylate substitution (dsC), and of hydrophobic group substitution (dsX)

| Polyanion | StructuraI features | | | | $EC_{50}$ (μg/mL [μM]) | |
|---|---|---|---|---|---|---|
| | *n* gly[a] | dsS | dsC | dsX | Binding[b] | $PrP_{res}$[c] |
| Size effect | | | | | | |
| $HM_1S_1$ | 1 | 1 | — | — | >100 [>700] | >100 [>700] |
| $HM_{60}S_{2.0}$ | 60 | 2.0 | — | — | >10.0 [>70.0] | 7.42 [0.378] |
| $HM_{250}S_{2.0}$ | 250 | 2.0 | — | — | 0.70 [0.008] | 0.09 [0.001] |
| $HM_{3000}S_{2.0}$ | 3000 | 2.0 | — | — | 0.09 [<0.001] | 0.10 [<0.001] |
| Sulfate effect | | | | | | |
| $HM_{250}S_{0.0}$ | 250 | 0.0 | — | — | >100 [>2.00] | 100 [>2.000] |
| $HM_{250}S_{0.3}$ | 250 | 0.3 | — | — | 7.10 [0.148] | 8.70 [0.181] |
| $HM_{250}S_{1.0}$ | 250 | 1.0 | — | — | 0.15 [0.002] | 0.01 [<0.001] |
| $HM_{250}S_{2.0}$ | 250 | 2.0 | — | — | 0.70 [0.008] | 0.09 [0.001] |
| $HM_{250}C_{0.5}S_{0.0}$ | 250 | 0.0 | 0.5 | — | nd | >10 [>0.100] |
| $HM_{250}C_{0.5}S_{0.5}$ | 250 | 0.5 | 0.5 | — | nd | >10 [>0.100] |
| $HM_{250}C_{0.5}S_{0.9}$ | 250 | 0.9 | 0.5 | — | nd | 8.93 [0.121] |
| $HM_{250}C_{0.5}S_{1.0}$ | 250 | 1.0 | 0.5 | — | nd | 0.39 [0.005] |
| $HM_{250}C_{0.5}S_{1.1}$ | 250 | 1.1 | 0.5 | — | nd | 3.35 [0.043] |
| $HM_{250}C_{0.5}S_{1.4}$ | 250 | 1.4 | 0.5 | — | nd | 9.40 [0.109] |
| Hydrophobic effect on $HM_{250}C_{0.5}S_{0.5}X_{0.3}$ | | | | | | |
| X = none | 250 | 0.5 | 0.5 | — | nd | >10 |
| X = -*n*-oct | 250 | 0.5 | 0.5 | 0.3 | nd | 8.5 |
| X = -eth-hex | 250 | 0.5 | 0.5 | 0.3 | nd | 3.0 |
| X = -*t*-oct | 250 | 0.5 | 0.5 | 0.3 | nd | 1.5 |
| X = -Phe-Me | 250 | 0.5 | 0.5 | 0.3 | nd | 0.6 |

$EC_{50}$ represents the concentration needed to inhibit 50% of PrPrec binding to immobilized heparin or 50% of PrP accumulation in cultured ScGT1-7 cells.
[a] *n* gly: number of glycosidic units.
[b] Concentration to inhibit 50% of PrPrec binding to immobilized haparin.
[c] Concentration to inhibit 50% of PrPres accumulation in cultured ScGT1-7 cells.

CCD black and video camera (Panasonic, WV-BL200, 12.5 mm objective) coupled to a Scion LG-3 image acquisition device. The amount of PrPres was determined by the ImageJ software according to the method described by Wayne Rasband at http://rsb.info.nih.gov/ij/docs/examples/dot-blot/index.html.

## Results and discussion

Heparan sulfates have recently emerged as cellular receptors implicated in cellular prion uptake [7,15]. These compounds, as other endogenous polysaccharides including heparin, CS and DS, have showed to bind prions [16,17] and to inhibit the neurotoxicity of amyloid fibrils [18]. On these bases, a number of polyanions have been tested for their capacities to inhibit PrPres accumulation. Here, the interaction of PrPrec with various polyanions including heparin, HS, CS-A, CS-B, CS-C, PPS, DNA, and the synthetic HM2602 and HM5004 were compared by using an ELISA based competition assay. The recombinant PrPrec used possesses the octarepeat basic epitope known to interact with heparin. In accord with previous reports, results in Fig. 2 show that PPS and Congo red bind to PrPrec better than to heparin, to HS, and to CS [19]. However, the two HMs showed the highest capacity to bind the protein while DNA remained the worst. It is important to remark that the tested products differ in a number of structural features including their sulfate, carboxylate and hydrophobic core contents and their molecular sizes (Table 1). This raises real questioning on the impact of these factors for compounds anti-prion activity. For instance, PPS, which PrPrec binding capacity was lower than that of the two HMs, is a 1–4 β-D-xylopyranoside of about 25 glycosidic units (25 gly/chain), highly sulfated (dsS = 2.0), and bearing 4-methylglucopyranosyluronic acids (dsC = 0.1, dsMethyl = 0.1) linked to C-2 positions every 10 xylopyranoses (Fig. 1) [20]. Compared to PPS, HM2602 is a 10 times longer polyanion (250 gly/chain in a 1–6 α-D-glucopyranoside backbone) bearing hydrophobic benzylamide residues (dsX = 0.2) and substituted with significantly lower sulfate and higher carboxylate contents (dsS = 0.7, dsC = 0.9) (Table 1). The HM5004 derivative differs from HM2602 not only in the absence of hydrophobicity but also in its degree of sulfation (dsS = 1.3) and carboxylation (dsC = 0.3) (Table 1). Hence, these results show the difficulty to find a logical relationship between these compounds structures and their PrPrec binding abilities.

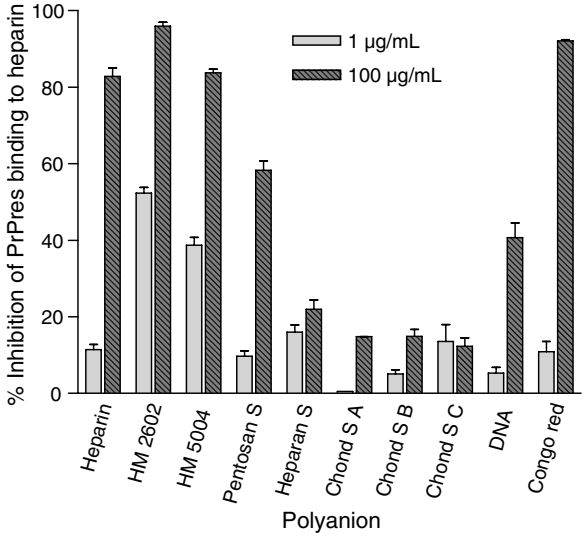


Fig. 2. Comparative effect of a series of polyanions on their capacities to inhibit PrPrec binding to immobilized heparin.

To examine the impact of these structural features on polyanions capacities to inhibit PrPrec binding to heparin and PrPres accumulation in ScGT1-7 infected cells, the work was continued with the HM family of compounds, in which size and substitution levels can be controlled. We first investigated the importance of the molecular length and prepared a series of products with equal sulfate content (dsS = 2.0) and different chain lengths including 60 ($HM_{60}S_{2.0}$), 250 ($HM_{250}S_{2.0}$), and 3000 ($HM_{3000}S_{2.0}$) gly/chain (Table 2). Products were tested by using mass concentrations (μg/mL) to compare them at equivalent number of glycosidic units and only examine the effect of the way these units are grouped. Results show that the polyanions abilities to inhibit the PrPrec binding to heparin increase by increasing the HMs length (Fig. 3A). This indicates that the polyanion PrP binding capacity by glycosidic unit is higher in long molecules possibly because the PrP binding sites can better be sheared in longer products. Interestingly, in the cell assay, a maximum effect at 250 gly/chain was observed. This might traduce a limited concentration of PrP in the cell environment or indicate that the biological activity is not only related to the polyanion PrP binding capacity but that other factors, as saturable receptors or other molecules binding PrP or heparin, can influence products efficacy. Obviously, the size effect was more apparent when transforming the μg/mL concentrations into μM concentrations (Table 2).

Studies in models involving or not prions have demonstrated that the content of sulfates in HMs is crucial for inducing biological activity and suggested that compounds activities increase with their sulfate contents [7,21]. In this work, a battery of HMs sized of 250 gly/chain ($HM_{250}$) but sulfated at different degrees (dsS = 0.0, 0.3, 1.0 and 2.0, corresponding to $HM_{250}S_{0.0}$, $HM_{250}S_{0.3}$, $HM_{250}S_{1.0}$, and $HM_{250}S_{2.0}$, respectively) were prepared. The ELISA based competition assay clearly showed that the HMs degree of sulfation (dsS) modifies, with a Gaussian shape (optimum dsS = 1.1), the capacities of HMs to inhibit PrPrec binding to immobilized heparin (Fig. 3C and D). The best effect was found with the partially sulfated $HM_{250}S_{1.0}$ and not, as could be expected, with the highly sulfated $HM_{250}S_{2.0}$. $HM_{250}S_{0.0}$ showed no effect. A similar Gaussian curve shape (optimum dsS = 1.0) was obtained when the effect of the carboxylated (dsC = 0.5) and differently sulfated products $HM_{250}C_{0.5}S_{0.0}$, $HM_{250}C_{0.5}S_{0.4}$, $HM_{250}C_{0.5}S_{0.9}$, $HM_{250}C_{0.5}S_{1.0}$, $HM_{250}C_{0.5}S_{1.2}$, and

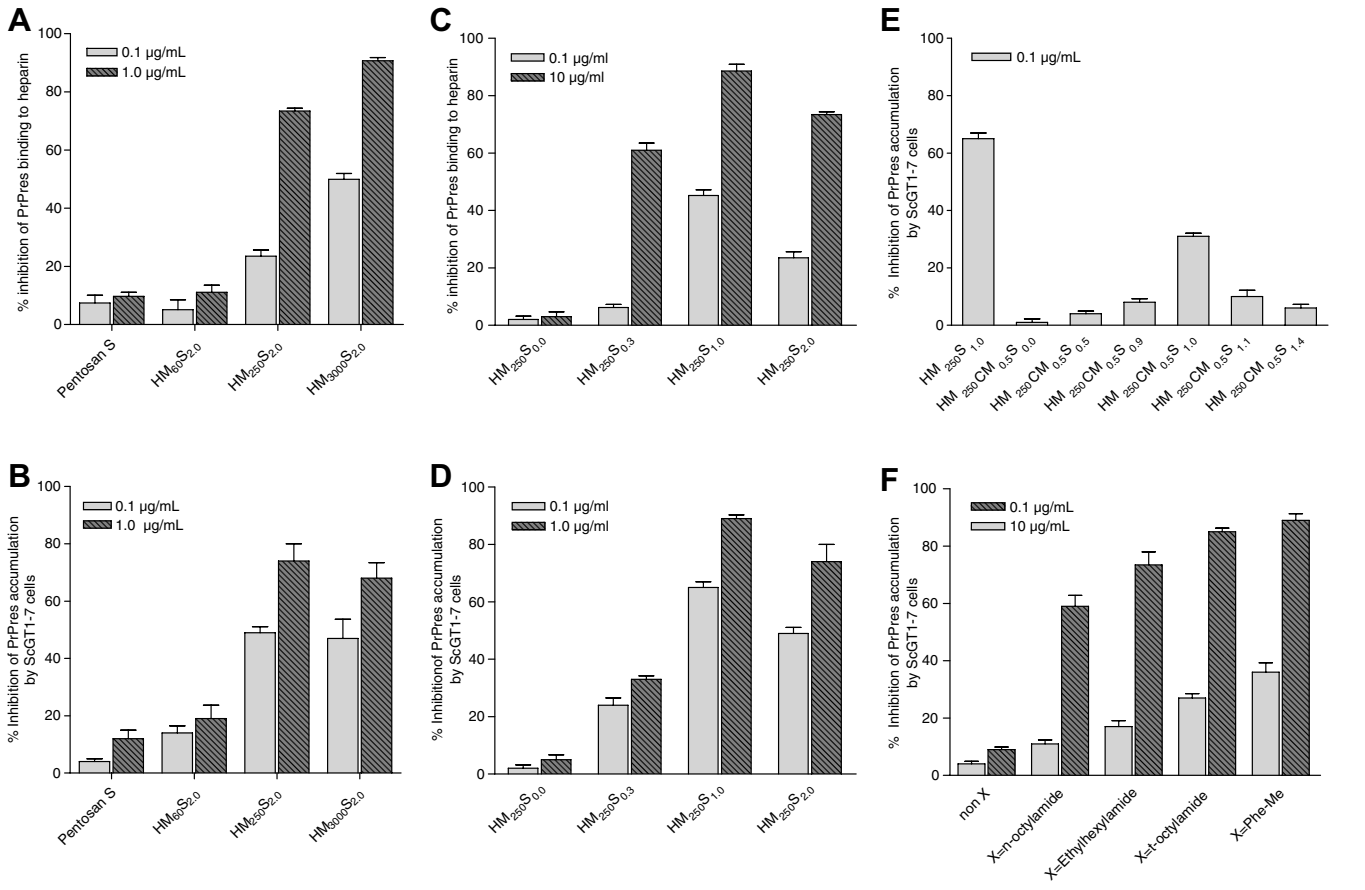


Fig. 3. Effect of the HMs structural features in the inhibition of PrPrec binding to immobilized heparin and of the PrPres accumulation in cultured ScGT1-7 cells. (A,B) Size effect; (C,D) sulfation degree effect; (E) sulfation degree and carboxymethyl effect; (F) hydrophobic substituent effect on $HM_{250}C_{0.5}S_{0.5}X_{0.3}$ products. For compounds structural details see Table 2.

$HM_{250}C_{0.5}S_{1.4}$ were compared in the cultured ScGT1-7 infected cells assay using the non-carboxylated $HM_{250}S_{1.0}$ as a control (Fig. 3E). Carboxymethyl groups were included considering that HMs, HS and other GAGs, are classically carboxylated (dsC = 0.5). These results indicate that the window range of sulfation for optimum effect is narrow and confirm our results with HM5004 (dsS = 1.3) which capacity to bind PrPrec was superior to that of PPS (dsS = 2) (Fig. 2), although the last is a smaller molecule. It has been demonstrated that sulfated polyanions interact with the heparin binding octapeptide-spanning 53–93 peptide motif of PrP [17], thus it is not surprising that the optimal dsS in HMs (dsS = 1.1) is as in heparin (dsS = 1.1) which efficacy compared to other polyanions might be lower due to its shorter size and to the presence of carboxylates. Conceivably, the presence of carboxylates in a polysaccharide must modify its global charge and its more stable conformation modifying its specific interaction sites with proteins [8]. Nonetheless, since a sole carboxylate content was used in this study the question concerning the influence of this feature on the anti-prion activity of polyanions remains to be reconsidered.

Finally, we investigated the role of HMs hydrophobicity on the ScGT1-7 cells assay. In previous studies, the presence of benzylamide moieties in HMs appeared to increase their anti-prion activities but the tested products differed also in their sulfate and carboxylate contents [7,9]. Here, we investigated if other considerably less toxic hydrophobic functionalities modify products efficacies. We prepared partially hydrophobized molecules with a low sulfated content (dsS = 0.5) and of intermediary size (250 gly/chain). The hydrophobic cores, noted as X in $HM_{250}C_{0.5}S_{0.5}X_{0.3}$, were *n*-octylamine (*n*-oct), *t*-octylamine (*t*-oct), ethylhexylamine (eth-hex), and phenylalanine methylester (Phe-Me). The X substitution degree (dsX) was 0.3 (dsX = 3, dsC = 0.5). Compared to the non-hydrophobized product $HM_{250}C_{0.5}S_{0.5}X_{0.0}$, $HM_{250}C_{0.5}S_{0.5}X_{0.3}$ showed increased abilities to inhibit PrPres accumulation in ScGT1-7 cells (Fig. 3F). It is to note that the more voluminous and aromatic PheMe derivative was the one presenting the best activity. This effect can be related to the observations suggesting that the C-terminal fragment of the PrPc is rich in hydrophobic residues which confer to the PrP an ideal binding point to hydrophobic moieties as observed with acridine derivatives [23,24]. This C-terminal domain has also been associated to the conformational change of PrPc into PrPres [25]. Thus, introduction of hydrophobic moieties in polyanions can be expected to increase their affinity for PrP and participate in the control of the conformational changes associated to pathogenesis, as suggested

by the HM2602 decrease of the protease-resistant PrPSc synthesis, without affecting the level of PrPc [25,26].

Taken together, our results suggest that there is a structural rational on HMs anti-prion activities. This rational can be used to analyse the activities of other polyanions and to design more efficient agents. Our binding experiments indicate that HMs can compete with cellular HS for interaction with prions and that this competition can be modulated by product size, degree of sulfation and carboxylation and by the introduction of hydrophobic moieties. This may have different consequences in the inhibition of the HS binding to the 37-kDa/67-kDa laminine receptor, which acts as a receptor for infectious prions [15] and in the inhibition of the PrPc conversion into PrPres [22], as showed in vitro for HS and other polyanions. We showed that increasing the polyanion size increases product efficacy. Although it might be considered in disagreement with a therapeutic application based in the compounds capacities to cross the gastrointestinal or the haematoencephalic blood brain barriers, the HMs nearly linear structures and the introduction of hydrophobicity should increase products bioavailabilities.

In conclusion, we propose that controlling the structure features of anti-prion agents can be at the base of more efficient therapeutic applications in prion diseases and should also contribute to progress on the understanding the physiopathology of these diseases. We are currently working in these areas.

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## References

[1] S.J. Collins, V.A. Lawson, C.L. Masters, Transmissible spongiform encephalopathies, Lancet 363 (2004) 51–61.
[2] P. Brown, Blood infectivity, processing and screening tests in transmissible spongiform encephalopathy, Vox Sang 89 (2005) 63–70.
[3] C. Weissmann, A. Aguzzi, Approaches to therapy of prion diseases, Annu. Rev. Med. 56 (2005) 321–344.
[4] S.B. Prusiner, Novel proteinaceous infectious particles cause scrapie, Science 216 (1982) 136–144.
[5] C.R. Trevitt, J. Collinge, A systematic review of prion therapeutics in experimental models, Brain 129 (2006) 2241–2265.
[6] A.D. Snow, T.N. Wight, D. Nochlin, Y. Koike, K. Kimata, S.J. DeArmond, S.B. Prusiner, Immunolocalization of heparan sulfate proteoglycans to the prion protein amyloid plaques of Gerstmann-Straussler syndrome, Creutzfeldt-Jakob disease and scrapie, Lab. Invest. 63 (1990) 601–611.
[7] L. Horonchik, S. Tzaban, O. Ben-Zaken, Y. Yedidia, A. Rouvinski, D. Papy-Garcia, D. Barritault, I. Vlodavsky, A. Taraboulos, Heparan sulfate is a cellular receptor for purified infectious prions, J. Biol. Chem. 280 (2005) 17062–17067.
[8] I. Capila, R.J. Linhardt, Heparin-protein interactions, Angew. Chem. Int. Ed. Engl. 41 (2002) 391–412.
[9] K.T. Adjou, S. Simoneau, N. Sales, F. Lamoury, D. Dormont, D. Papy-Garcia, D. Barritault, J.P. Deslys, C.I. Lasmezas, A novel generation of heparan sulfate mimetics for the treatment of prion diseases, J. Gen. Virol. 84 (2003) 2595–2603.
[10] A. Mange, O. Milhavet, H.E. McMahon, D. Casanova, S. Lehmann, Effect of amphotericin B on wild-type and mutated prion proteins in cultured cells: putative mechanism of action in transmissible spongiform encephalopathies, J. Neurochem. 74 (2000) 754–762.
[11] D. Papy-Garcia, V. Barbier-Chassefière, V. Rouet, M.E. Kerros, C. Klochendler, M.C. Tournaire, D. Barritault, J.P. Caruelle, E. Petit, Nondegradative sulfation of polysaccharides. Synthesis and structure characterization of biologically active heparan sulfate mimetics, Macromolecules 38 (2005) 4647–4654.
[12] S. Najjam, R.V. Gibbs, M.Y. Gordon, C.C. Rider, Characterization of human recombinant interleukin 2 binding to heparin and heparan sulfate using an ELISA approach, Cytokine 9 (1997) 1013–1022.
[13] I. Barbosa, S. Garcia, V. Barbier-Chassefiere, J.P. Caruelle, I. Martelly, D. Papy-Garcia, Improved and simple micro assay for sulfated glycosaminoglycans quantification in biological extracts and its use in skin and muscle tissue studies, Glycobiology 13 (2003) 647–653.
[14] D.A. Kocisko, G.S. Baron, R. Rubenstein, J. Chen, S. Kuizon, B. Caughey, New inhibitors of scrapie-associated prion protein formation in a library of 2000 drugs and natural products, J. Virol. 77 (2003) 10288–10294.
[15] S. Gauczynski, D. Nikles, S. El-Gogo, D. Papy-Garcia, C. Rey, S. Alban, D. Barritault, C.I. Lasmezas, S. Weiss, The 37-kDa/67-kDa laminin receptor acts as a receptor for infectious prions and is inhibited by polysulfated glycanes, J. Infect. Dis. 194 (2006) 702–709.
[16] B. Caughey, K. Brown, G.J. Raymond, G.E. Katzenstein, W. Thresher, Binding of the protease-sensitive form of PrP (prion protein) to sulfated glycosaminoglycan and congo red [corrected], J. Virol. 68 (1994) 2135–2141.
[17] R.G. Warner, C. Hundt, S. Weiss, J.E. Turnbull, Identification of the heparan sulfate binding sites in the cellular prion protein, J. Biol. Chem. 277 (2002) 18421–18430.
[18] M. Perez, F. Wandosell, C. Colaco, J. Avila, Sulphated glycosaminoglycans prevent the neurotoxicity of a human prion protein fragment, Biochem. J. 335 (Pt 2) (1998) 369–374.
[19] B. Caughey, G.J. Raymond, Sulfated polyanion inhibition of scrapie-associated PrP accumulation in cultured cells, J. Virol. 67 (1993) 643–650.
[20] M. Degenhardt, P. Ghosh, H. Watzig, Studies on the structural variations of pentosan polysulfate sodium (NaPPS) from different sources by capillary electrophoresis, Arch. Pharm. (Weinheim) 334 (2001) 27–29.
[21] D. Ledoux, D. Papy-Garcia, Q. Escartin, M.A. Sagot, Y. Cao, D. Barritault, J. Courtois, W. Hornebeck, J.P. Caruelle, Human plasmin enzymatic activity is inhibited by chemically modified dextrans, J. Biol. Chem. 275 (2000) 29383–29390.
[22] N.R. Deleault, J.C. Geoghegan, K. Nishina, R. Kascsak, R.A. Williamson, S. Supattapone, Protease-resistant prion protein amplification reconstituted with partially purified substrates and synthetic polyanions, J. Biol. Chem. 280 (2005) 26873–26879.
[23] M. Horiuchi, G.S. Baron, L.W. Xiong, B. Caughey, Inhibition of interactions and interconversions of prion protein isoforms by peptide fragments from the C-terminal folded domain, J. Biol. Chem. 276 (2001) 15489–15497.
[24] B. Caughey, G.S. Baron, Factors affecting interactions between prion protein isoforms, Biochem. Soc. Trans. 30 (2002) 565–569.
[25] S.A. Priola, J. Chabry, K. Chan, Efficient conversion of normal prion protein (PrP) by abnormal hamster PrP is determined by homology at amino acid residue 155, J. Virol. 75 (2001) 4673–4680.
[26] O. Kovalchuk, S. Tzaban, Y. Tal, L. Horonchik, J.D. Esko, I. Vlodavsky, A. Taraboulos, Cellular heparan sulfate participates in the metabolism of prions, J. Biol. Chem. (2003).