Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 363 (2007) 95–100
BBRC
www.elsevier.com/locate/ybbrc

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

Mohand-Ouidir Ouidja [a,b,c,1], Emmanuel Petit [d,1,2], Marie-Emmanuelle Kerros [a,d,3], Yasunori Ikeda [a], Christophe Morin [a], Gilles Carpentier [a], Denis Barritault [a,d], Jeanne Brugère-Picoux [c], Jean-Philippe Deslys [b], Karim Adjou [b,c], Dulce Papy-Garcia [a,d,*]

[a] *Laboratoire CRRET CNRS-UMR 7149, Université Paris 12, 94010 Créteil, France*
[b] *Ecole Nationale Vétérinaire d'Alfort, Laboratoire de Pathologie du Bétail, 94704 Maisons-Alfort, France*
[c] *CEA, Institute of Emerging Diseases and Innovative Therapies, 92265 Fontenay-aux-Roses, France*
[d] *OTR3, 4, Rue Française, F75001 Paris, France*

Received 7 August 2007
Available online 29 August 2007

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

* Corresponding author. Address: Laboratoire CRRET CNRS-UMR 7149, Université Paris 12, 94010 Créteil, France. Fax: +33 145171816.
E-mail address: papy@univ-paris12.fr (D. Papy-Garcia).
[1] These authors equally contributed to this work.
[2] Present address: Laboratoire des glucides EMPV CNRS-UMR 6219, IUT-GB, UPJV, Avenue des Facultés, 80025 Amiens, France.
[3] Present address: Laboratoire Océanographique de Villefranche CNRS-UMR 7093, Villefranche-sur-Mer, France.

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.08.113

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.

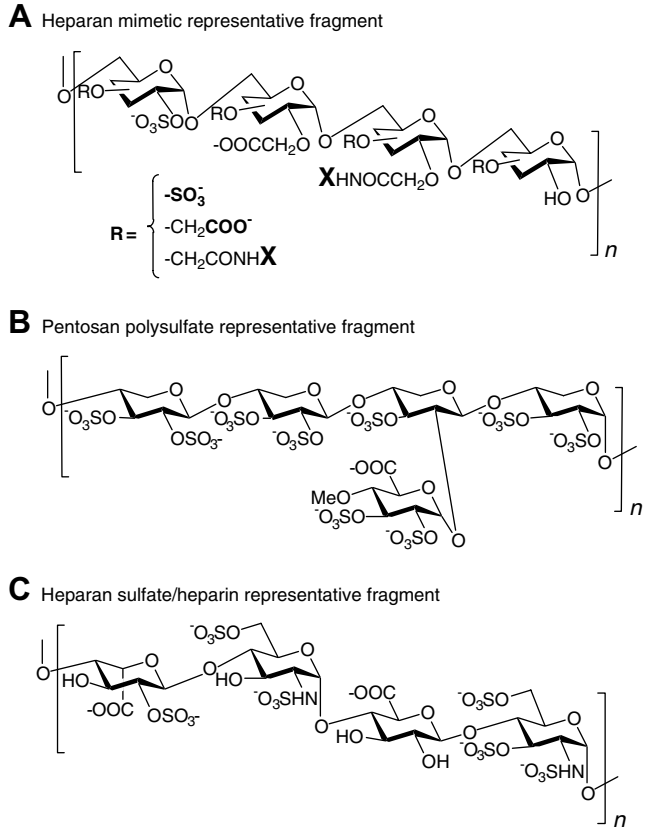

Fig. 1. General formula of representative fragments of sulfated polysaccharides. The HS fragment is here represented by a highly sulfated sequence.

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