Sulfated Glycopolymer Thin Films Preparation, Characterization and Biological Activity

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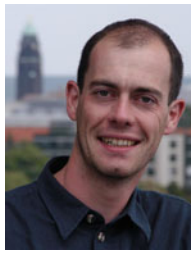

Introduction

- blood coagulation is one of the body responses after blood is contacting a foreign material and may lead to thrombus formation (Fig. 1)
- heparin, a polyanionic glycosaminoglycan (GAG) (Fig. 2), catalyzes the AT mediated serine protease inhibition
- chain length and sulfation degree of GAGs influence the inhibition of Thrombin and Factor Xa (FXa) by AT [1-5]
- fully sulfated synthetic glycopolymers show anticoagulant activity [6]
- accelerated AT inhibition is related to the charge density of synthetic polyanions [7,8]
- sulfonate density and carboxylate/sulfonate ratio determine the anticoagulant activity of solid beads [9-11]

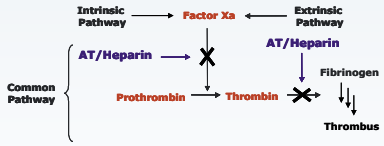

Fig. 1: Simplified coagulation cascade and antithrombin (AT) mediated inhibition of thrombin/Factor Xa

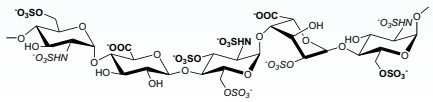

Fig. 2: Pentasccharide unit of the naturally occurring anticoagulant heparin

Strategy

- to explore the structural requirements of synthetic polymer architectures to mimic heparinoid behavior
- to introduce glycosidic structures and sulfate groups to enhance the anticoagulant characteristics of the maleic anhydride copolymer (MA) films (Fig. 3)
- to study the surface characteristics after film alteration by means of film thickness, chemical composition, wettability and morphology

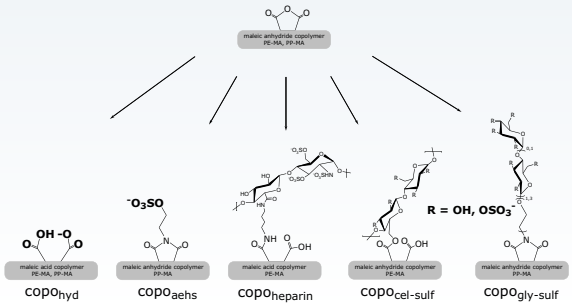

Fig. 3: Schematic presentation of modified maleic anhydride copolymer thin films

Results and Discussion

glycoside synthesis and surface modification

- spacer synthesis followed a published procedure [12]
- BF_3 etherate promoted glycosylation resulting in β-glycosides (Fig. 4)
- deprotected glycosides obtained after transesterfication
- Pd/C catalyzed hydrogenation converted the azides into amines **01**-**04** (Fig. 5)

- immobilization of AEHS (15 mM, pH 10), the glycosides **01**-**04** (10 mM, pH 8) and cellulose (DP ~ 230,1300; 0.5 wt% NMMO) onto MA thin films
- sulfation of the glycosidic copolymer films

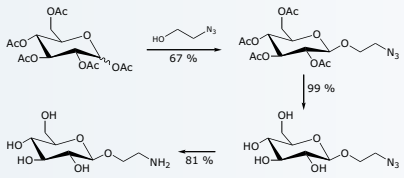

Fig. 4: Exemplary reaction for glycoside preparation

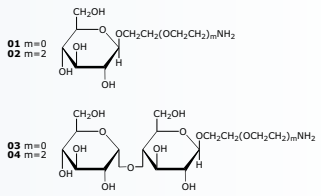

Fig.5: Synthesized glycosides used for immobilization

surface characterization

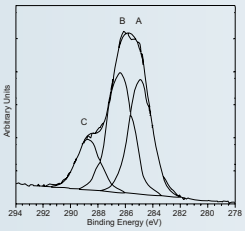

Fig. 6: XPS C1s spectrum of glycopolymers (A) CH, 285 eV; (B) C-OH, C-O-C, 286.5 eV; (C) C=O, 289.4 eV

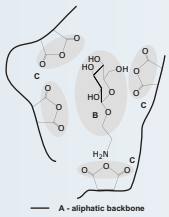

Fig. 7: Schematic presentation of reacting sites and XPS C1s labels

Tab.1: Surface properties of the sulfated copolymer layers

copo sample	film growth in nm	S2p in atomic %	stat. contact angle in dgr	surface roughness in nm
PP-MA	2.8 ± 0.3	-	71 ± 2	-
AEHS	1.7 ± 0.2	2.0	35 ± 2	-
01-sulf	2.2 ± 0.2	1.3	59 ± 1	0.2
02-sulf	2.6 ± 0.2	0.98	63 ± 2	-
03-sulf	3.0 ± 0.2	1.02	41 ± 1	0.2
04-sulf	3.6 ± 0.6	1.5	58 ± 1	-
PE-MA	6.3 ± 1.1	-	72 ± 3	-
cel-sulf I	8.4 ± 0.1	2.5	43 ± 3	2.4
cel-sulf II	14.5 ± 0.1	1.8	49 ± 1	2.3
heparin	5.4 ± 5.4	< 0.1	-	-

Tab.2: Surface density fit and sulfate/copolymer ratio

copo sample	grafting density in %	sulfate/copolymer unit ratio in %
AEHS	42	42
01-sulf	67	37
02-sulf	43	24
03-sulf	43	27
04-sulf	52	48

- glycopolymer layers obtained by immobilization of glycosides from different solutions (1-30 mM, pH 8) were studied by XPS (Fig. 6)
- XPS and ellipsometry (data not shown) proved incorporation of the glycosides into the film (Fig. 7)
- intra-/intermolecular cross-linkings, electrostatic interactions and rearrangement of the chains contributed to film thickness

- surface analysis after sulfation (Tab. 1)
- 5 wt% SO_3*NMe_3/DMF, 90 °C, 3 h was found to be optimal
- stable films were obtained as proven by ellipsometry
- the sulfur content was measured by XPS
- smooth and more hydrophilic surfaces compared to pure copolymer films were produced

- N, Si, S atomic % and data from C1s peak – aliphatic carbon, COH, COOH atomic % - were taken to fit the surface densities of the immobilized molecules (Tab. 2)
- obtained grafting densities were used to calculate the sulfate/copolymer ratio

biological surface activity

- investigation of the surface bound AT using FITC labelled anti-human AT
- fluorescence intensities measured by cLSM are shown in Fig.8
- highest intensities were found for the heparin/albumin-based references HMW/LMW [13]
- cel-sulf I bound more AT due to a higher sulfation degree compared to cel-sulf II
- despite the higher sulfur content of the AEHS sample, lower amount of AT was bound in comparison to the heparinized surface
- glycosidic structures seem to be more important than solely sulfate groups
- more hydrophilic mono-/diglycosidic samples (**01**, **03**) have higher affinity than **02** and **04**, respectively
- less AT bound to polymer films devoid of saccharide moieties

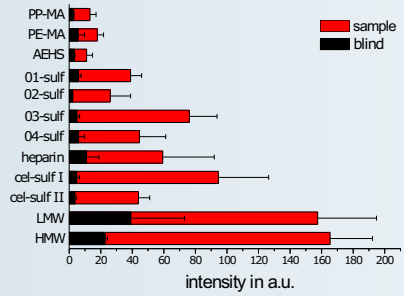

Fig. 8: Fluorescence intensities of FITC labelled samples

Conclusion

- amino functionalized mono-/diglycosides were synthesized
- sulfated polymeric supports were obtained after modification of the MA copolymer thin films
- an incorporation of the agents into the copolymer layer was concluded from surface analysis data (Fig. 9)
- combination of glycosidic structures and sulfate groups resulted in greater AT affinity
- at polyanionic charges not associated with glycosidic structures less AT binding was observed
- current investigations on Thrombin/FXa activities and whole blood incubation indicate a reduced activation of coagulation

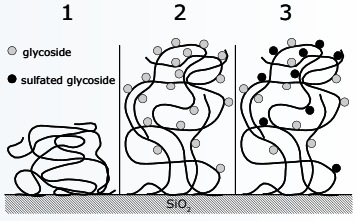

Fig. 9: Sulfation of immobilized glycopolymers

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[01] Holmer, E.; Kurachi, K.; Söderström, G. Biochem. J. 1981, 193, 395-400.
[02] Toida, T.; Chaidedgumjorn, A.; Lindhardt, R. J. Trends Glycosci. Glycotechn. 2003, 15, 29-46.
[03] Sissi, C.; Naggi, A.; Torri, G.; Palumbo, M. Seminars in Thrombosis and Hemostasis 2001, 27, 483-487.
[04] Maaroufi, R. M.; Jozefowicz, M.; Tapon-Bretaudiére, J.; Fischer, A.-M. Carbohydr. Res. 2006, 341, 672-676.
[05] Vongchan, P.; Sajomsang, W.; Subyen, D.; Kongtawelert, P. Carbohydr. Res. 2002, 337, 1233-1236.
[06] Sun, X.-L.; Grande, D.; Baskaran, S.; Hanson, S. R.; Chaikof, E. L. Biomacromolecules 2002, 3, 1065-1070.
[07] Linhardt R.J., Toida T., Carbohydrates as Drugs, Heparin Analogs-Development and Applications, Marcel Dekker,1997, 277
[08] Monien, B. H.; Desai, U. R. J. Med. Chem. 2005, 48, 1269-1273.
[09] Mauzac, M.; Aubert, N.; Jozefonvicz, J. Biomaterials 1982, 3, 221-224.
[10] Kanmangne, F. M.; Labarre, D.; Serne, H.; Jozefowicz, M. Biomaterials 1985, 6, 297-302.
[11] Douzon, C.; Kanmangne, F. M.; Serne, H.; Labarre, D.; Jozefowicz, M. Biomaterials 1987, 8, 190-194.
[12] Chernyak, A. Y.; Shamrat, G. V. M.; Kononov, L. .; Rao, A. V. R. Carbohydr. Res. 1992, 223, 303-309.
[13] Brynda, E.; Houska, M.; Jirousková, M.; Dyr, J. E. J. Biomed. Mater. Res. 2000, 51, 249-257.