Supercharged Fluorescent Protein as a Versatile Probe for the Detection of Glycosaminoglycans in Vitro and in Vivo

ARTICLE *in* ANALYTICAL CHEMISTRY · AUGUST 2015

Impact Factor: 5.64 · DOI: 10.1021/acs.analchem.5b02071 · Source: PubMed

6 AUTHORS, INCLUDING:

Wen Jun Han

Shandong University

15 PUBLICATIONS **75** CITATIONS

SEE PROFILE

Fuchuan Li

Shandong University

16 PUBLICATIONS **433** CITATIONS

SEE PROFILE

analytical chemistry

Article

pubs.acs.org/ac

Supercharged Fluorescent Protein as a Versatile Probe for the Detection of Glycosaminoglycans *in Vitro* and *in Vivo*

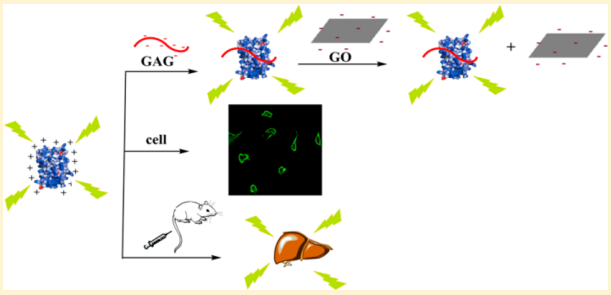
Wenshuang Wang,[†] Naihan Han,[†] Ruijuan Li,[‡] Wenjun Han,[†] Xiaoru Zhang,*[,‡] and Fuchuan Li*[,†]

[†]National Glycoengineering Research Center and State Key Laboratory of Microbial Technology, Shandong University, Jinan, Shandong 250100, P. R. China

[‡]Key Laboratory of Sensor Analysis of Tumor Marker, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao, Shandong 266042, P. R. China

Supporting Information

ABSTRACT: Glycosaminoglycans (GAGs) are linear acidic heteropolysaccharides that are ubiquitously expressed in animal tissues and participate in various life processes. To date, the detection and visualization of GAGs in complex biological samples and living organisms remain a challenge because of the lack of powerful biocompatible probes. In this study, a superpositively charged green fluorescent protein (ScGFP) was shown great potential in GAG detection for the first time. First, on the basis of the phenomenon of GAGs dose-dependently inhibiting the fluorescence quenching of ScGFP by graphene oxide, a simple and highly sensitive signal-on homogeneous platform was established for detecting and quantifying GAGs, even in complex samples such as heparin in citrated plasma and oversulfated chondroitin sulfate in heparin. Furthermore, ScGFP with excellent stability and biocompatibility could be easily used as a highly sensitive and selective probe to visualize different types of GAGs *in vitro* and *in vivo* through combination with specific GAG-degrading enzymes. This study introduces a versatile probe for GAG detection, which is easy to prepare and which shows a high practical value in basic research and medical applications.



Glycosaminoglycans (GAGs) are linear, negatively charged heteropolysaccharides that are ubiquitously expressed on cell surfaces, in extracellular matrices, and in basement membranes of animals. GAGs not only have structural roles in connective tissues but also participate in various important physiological and pathological processes.[1−7] These biological functions promote GAGs as important therapeutic reagents or targets in clinical and pharmaceutical fields.[8] The three most abundant classes of GAGs in animals are hyaluronan (HA), heparan sulfate (HS)/heparin (Hep), and chondroitin sulfate (CS)/dermatan sulfate (DS). The three major classes of GAGs are composed of repeating disaccharide units of hexuronic acid and hexosamine.[9] HA is the simplest GAG that neither contains sulfo groups nor is attached to a core protein. In contrast, HS/Hep and CS/DS are covalently attached to core proteins to form proteoglycans (PGs), and these GAGs are modified by sulfation and epimerization. These modifications introduce enormous microheterogeneity to the GAG chains that usually have key roles in various biological functions through interacting with specific proteins. However, structural complexity and diversity present a huge challenge to structural and functional studies of GAGs.

Various cationic dyes, such as alcian blue, toluidine blue, and amido black, have been widely used for the detection and visualization of GAGs in biological samples. However, such dyes have a number of shortcomings including poor biocompatibility, which is not suitable for intravital staining, poor selectivity, which usually causes a high background, and low sensitivity. Recently, a variety of synthetic cationic chromophores have been developed for the high-sensitivity detection of Hep in buffer or serum.[10−15] Nevertheless, a biocompatible, sensitive, and versatile probe is urgently needed for the detection of GAGs, notably in complex samples.

More recently, a kind of superpositively charged green fluorescent protein (ScGFP) was developed by genetic mutation[16] and used as an effective vehicle for protein and nucleic acid delivery into mammalian cells[17,18] and as a versatile probe for nucleic acid detection and epigenetics analysis.[19−21] Using a ScGFP variant with a net charge of +36 as a model, we demonstrate that ScGFP is a powerful and versatile probe for detecting GAGs in various important applications, including the highly sensitive quantification of Hep in citrated plasma and oversulfated chondroitin sulfate (OSCS) contaminants in Hep and the visualization of GAGs on the surface of living cells and in organs.

Received: May 25, 2015
Accepted: August 19, 2015
Published: August 19, 2015

DOI: 10.1021/acs.analchem.5b02071
Anal. Chem. 2015, 87, 9302−9307

EXPERIMENTAL SECTION

Materials. Standard CS/DS and HS/Hep unsaturated disaccharides were purchased from Iduron (Manchester, UK). DS from porcine skin was obtained from Seikagaku Corp. (Tokyo, Japan). Cyanoborohydride ($NaBH_3CN$), 2-aminobenzamide (2-AB), CS-A from bovine trachea, heparinase (Hepase) I (EC 4.2.2.7), II (EC 4.2.2.7), and III (EC 4.2.2.8), Chondroitinase ABC (CSase ABC) (EC 4.2.2.4), Hep from porcine intestinal mucosa, HA from bovine vitreous humor, and avidin were obtained from Sigma. Fetal bovine serum (FBS) was obtained from Hyclone. Graphene oxide (GO) was obtained from XF Nano Inc. (Nanjing, China). ScGFP and StGFP were expressed and purified as described in the Supporting Information. Human citrated plasma was obtained from volunteers. All other chemicals and reagents were of the highest quality available. Stock solutions of ScGFP, Hep, and GO were prepared using Tris-HCl buffer (10 mM Tris-HCl, 100 mM NaCl, and pH 7.4).

Quantification of GAGs in Buffer and Plasma. Different amounts of GAG stock solution (2.5 mg/mL) were added to 100 μL of ScGFP (5 μg/mL) and then incubated for 30 min at room temperature. In total, 10 μL of GO (1 mg/mL) and an appropriate amount of Tris-HCl buffer (10 mM Tris-HCl, 100 mM NaCl, and pH 7.4) were subsequently added to produce a final volume of 200 μL. After another 30 min, the mixture was subjected to fluorescence measurements.

To detect heparin in plasma, the Hep (2.5 mg/mL), ScGFP (5 μg/mL), and GO (1 mg/mL) stock solutions were prepared using Tris-HCl buffer (10 mM Tris-HCl, 100 mM NaCl, and pH 7.4) with 10% citrated plasma. The procedures for measuring Hep in 10% citrated plasma were identical to those in the buffer mentioned above.

Detection of Oversulfated Chondroitin Sulfate in Heparin. Heparin (10 μg) containing 10% of other types of GAGs (such as HA, CS, DS, or OSCS) was digested with Hepases (I, II, and III, 5 mU each) or with the corresponding inactive enzymes in 20 μL of Tris-HCl buffer (25 mM Tris-HCl, 10 mM $CaCl_2$, and pH 7.0) at 30 °C for 4 h. The digest (8 μL) was mixed with 100 μL of ScGFP (5 μg/mL) in 10 mM Tris-HCl containing 100 mM NaCl. The mixture was incubated for 30 min at room temperature. Finally, 10 μL of GO (1 mg/mL) and 82 μL of Tris-HCl buffer (10 mM Tris-HCl, 100 mM NaCl, and pH 7.4) were added to the mixture to make a final volume of 200 μL. After another 30 min, the mixture was subjected to fluorescence measurements. Then, heparin (10 μg) containing different amounts of OSCS was used for the same experiment.

Visualization of GAGs on the Surface of Living Cells. Lung cancer A549 cells were plated in a 35 mm dish with a 14 mm glass bottom well (Shengyou Biotechnology, Hangzhou, China). After plating for 24 h, the cells were washed three times with PBS and were then treated with CSase ABC, Hepases (I and III), or both CSase ABC and Hepases. The treated cells were incubated with 200 μL of ScGFP (10 μg/mL) for 5 min at room temperature, and unbound ScGFP was immediately removed by washing three times with PBS. After the cell nuclei were stained by directly adding DAPI, the cells were visualized using a scanning laser microscope LSM 700 (Carl Zeiss Inc., Germany) with a Zeiss LSM Image Browser.

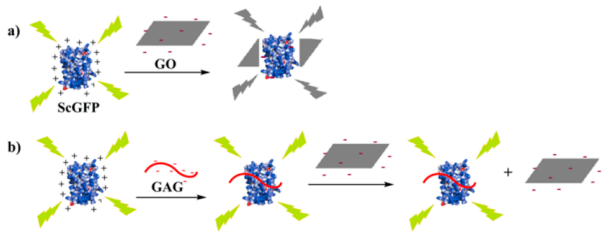
For the FACS assay, 293T cells were seeded in 60 mm tissue culture plates (NUNC) containing 5 mL of DMEM supplemented with 10% fetal bovine serum (FBS, Hyclone). After 24 h, cells were suspended by pipetting gently, collected by centrifuging for 10 min at 1200 rpm, and treated with enzymes. The enzyme-treated cells were then incubated with 200 μL of ScGFP (10 μg/mL) for 5 min at room temperature, washed with PBS, and resuspended in PBS. Fluorescence intensities of cells were acquired with a FACS Aria III flow cytometer (Becton Dickinson, U.S.A). Data were analyzed with Flowjo (Tree Star Inc., U.S.A.).

Visualization of GAGs *in Vivo*. To visualize the different types of GAGs in organs, PBS, CSase ABC (50 mU), Hepases (I and III, 25 mU each), or both CSase ABC (50 mU) and Hepases (50 mU) were injected into the Balb/c mouse via the tail vein. After 1 h, 60 μg of ScGFP was injected into each mouse through the tail vein. After another 30 min, the organs of the mouse were removed and photographed by a fluorescence imager (Fluor Chem Q, CELL biosciences).

RESULTS AND DISCUSSION

As a positively charged protein, ScGFP can interact with hydroxyl, epoxy, and carboxyl groups of GO through electrostatic and hydrogen bonding attraction,[22−24] which can lead to fluorescence quenching of ScGFP (route a in Scheme 1). As shown in Figure S1, the fluorescence intensity decreased significantly with the addition of GO, indicating an energy/charge transfer between ScGFP and GO. Similarly, ScGFP can also bind to the carboxylate, sulfate, and hydroxyl groups of GAGs through electrostatic and hydrogen bonding interactions.[25] The presence of GAGs can impede the fluorescence quenching of ScGFP by GO (route b in Scheme 1). Thus, this fluorescence intensity increase could be exploited as an assay for determining the concentration of GAGs. This sensing platform provides a signal-on assay and a simple and sensitive method for the detection of GAGs.

Scheme 1. Schematic Illustration of the ScGFP-GO Platform for GAG Detection[a]



[a](a) In the absence of GAGs; (b) in the presence of GAGs.

Hep is one of the most well-known GAGs; it is widely used as a clinical anticoagulant during surgery and as an anticoagulant therapy. However, Hep overdose often causes serious complications such as hemorrhages and thrombocytopenia. To avoid such Hep-induced side effects, developing simple and reliable methods for the detection of Hep in serum or plasma has always been an active topic.[15,26−30] Herein, the proposed sensing platform of ScGFP-GO was tested for the quantification of Hep. Under optimal conditions (see Figures S2−S6), the fluorescence intensity increased with increasing Hep concentration in the range from 0.05 to 1 μg/mL (Figure 1A), corresponding to 3.3−66.7 nM. This method is compared with other methods shown in Table S1. Notably, the sensitivity of such platform for heparin detection is similar to the near-infrared fluorescence assay using trypsin stabilized gold

DOI: 10.1021/acs.analchem.5b02071
Anal. Chem. 2015, 87, 9302−9307

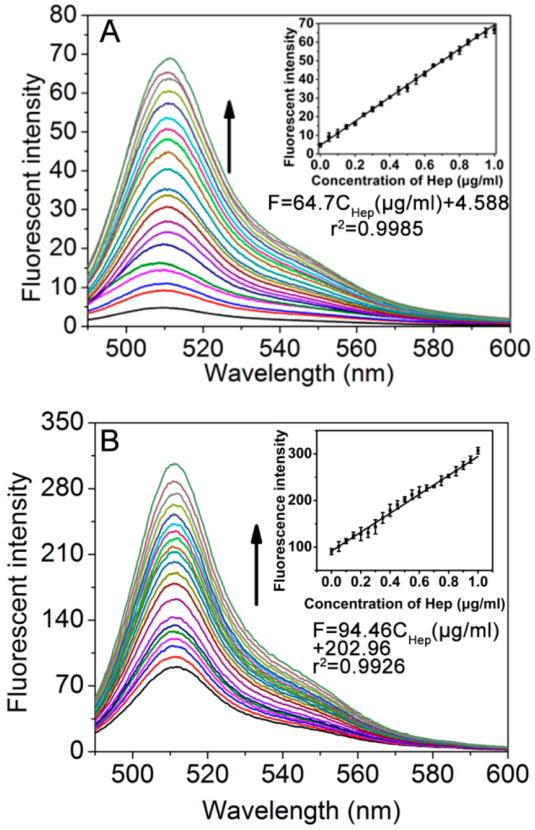


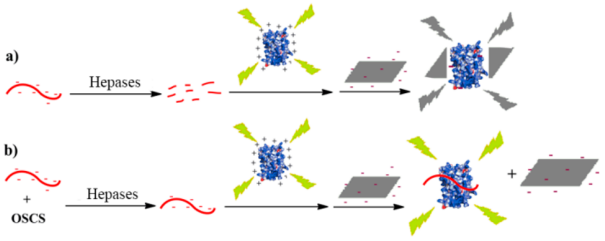
Figure 1. Fluorescence emission spectra of the ScGFP-GO conjugate in the presence of different concentrations of Hep in (A) Tris-HCl buffer (10 mM Tris-HCl, 100 mM NaCl, and pH 7.4) and (B) Tris-HCl buffer containing 10% citrated plasma. From bottom to top: 0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, and 1 μg/mL. The final concentrations of ScGFP and GO were 2.5 and 50 μg/mL, respectively. The inset displays the corresponding calibration curve.

nanoclusters (0.1−4.0 μg/mL),[28] fluorescence detection based on quantum dots and a functional ruthenium polypyridyl complex (0.32−1.15 μg/mL),[27] and a phloxine B/polyethylenimine system-based optical sensing platform (0.12−18.7 μg/mL),[31] much better than an anion-exchange-based amperometric assay using polyimidazolium as synthetic receptor (7.5−150 μg/mL)[29] but inferior to surface-enhanced Raman scattering sensing based on antiaggregation of functionalized silver nanoparticles (5×10^{-4} to 0.15 μg/mL).[30] Moreover, this sensing platform could be applied to determine HA, CS, and DS (Figure S8).

To further demonstrate the feasibility of this approach in practical clinical analyses, the detection of Hep in real biological samples was investigated. The prepared sensor was also carried out in Tris-HCl buffer (10 mM, 100 mM NaCl, and pH 7.4) containing 10% citrated plasma. As shown in Figure 1B, the background in the diluted citrated plasma was higher than that in the pure buffer solution, which may be caused by the interference of endogenous GAGs, nucleic acids, and the competition of some proteins. However, the detection can still perform well in such biological media because a positive correlation between the fluorescence intensity and Hep concentration from 0.05 to 1 μg/mL (3.3−66.7 nM) was noted. This sensitivity in complex matrices is similar to some recent reports.[26,27,32] The analysis of Hep in diluted citrated plasma indicated the availability of the proposed method for the detection of GAGs in complex biological samples.

Contamination of the heparin with OSCS has resulted in adverse effects and at least 149 deaths in several countries during the 2007−2008 period.[33−35] Therefore, the development of methods to detect OSCS in Hep has become another active issue.[36−41] In this study, the ScGFP-GO platform was further used to detect OSCS in Hep in combination with heparinases (Hepases) digestion. The principle for the detection of OSCS in Hep is shown in Scheme 2. In the absence of OSCS, Hep can be digested into short fragments by Hepases (I, II, and III), and thus, the fluorescence of ScGFP can be fairly quenched by GO without the competition of Hep. However, in the presence of OSCS, which can strongly inhibit Hepase activity,[42] the retained long Hep chains can prevent the interaction between ScGFP and GO, which leads to the preservation of ScGFP fluorescence. The enzyme activity will decline with increases in the OSCS concentration. Thus, the fluorescence intensity correlated with the amount of OSCS. As shown in Figure 2, when serially diluted OSCS contaminants ranging from 10 to 10^{-9} wt % were added to 10 μg Hep solutions, the fluorescence intensity increased gradually with the increase of the OSCS concentration, which implied that more and more undigested Heps were left. Compared with the OSCS-free Hep solution, a significant increase in fluorescent intensity was noted even in the presence of 10^{-6} wt % OSCS,

Scheme 2. Schematic Illustration for the Fluorescence Detection of OSCS Contaminants in Hep



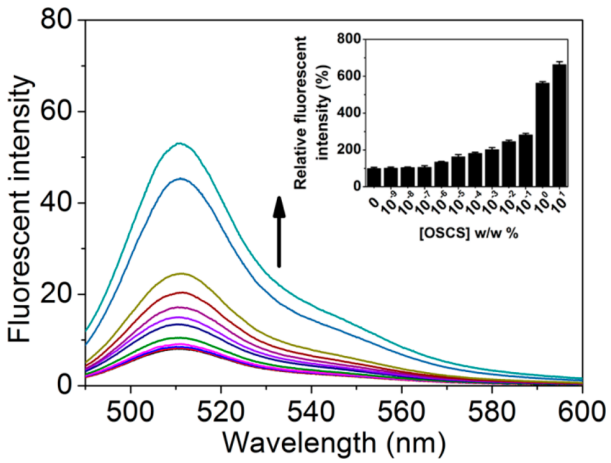


Figure 2. Fluorescence intensity of the ScGFP-GO platform using the Hepases-treated Hep solution spiked with different amounts of OSCS. The content of OSCS in Hep (wt %) from bottom to top is the following: 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 10^{0}, and 10^{1}. The inset displays the relative fluorescent intensity of ScGFP-GO after adding Hep containing different amounts of OSCS (wt %).

DOI: 10.1021/acs.analchem.5b02071
Anal. Chem. 2015, 87, 9302−9307

which is more sensitive than most previous reports[36−40] and only inferior to the assay performed with an Au-Hep-dye nanosensor. However, the preparation of that nanosensor required a relatively complex synthetic procedure.

In addition, the influence of other natural glycosaminoglycan impurities was also measured to evaluate the selectivity of the proposed method for OSCS contaminants. As shown in Figure S10, after enzyme treatment, the fluorescence of Hep with 10% HA, CS-A, and DS was substantially quenched, whereas the fluorescence of Hep with 10% OSCS slightly changed. Therefore, the developed method had a high sensitivity and selectivity and can be used to detect trace amounts of OSCS contaminants in Hep and to monitor drug quality.

Various PGs, such as glypicans and syndecans, are expressed on almost all cell surfaces in animals. The sulfated GAG side chains (e.g., HS and/or CS) of these PGs constitute a major source of macromolecular polyanions that surround cells. Considering the fact that ScGFP has a high net positive charge and has a good capacity to deliver proteins and genes into cells, this protein may interact with cell surface GAGs and can thus be used as a biocompatible probe to detect GAGs on the surfaces of living cells directly without the assistance of GO. As shown in Figure 3, the cell surfaces of living A549 cells could be specifically stained by ScGFP after a short incubation of 5 min.

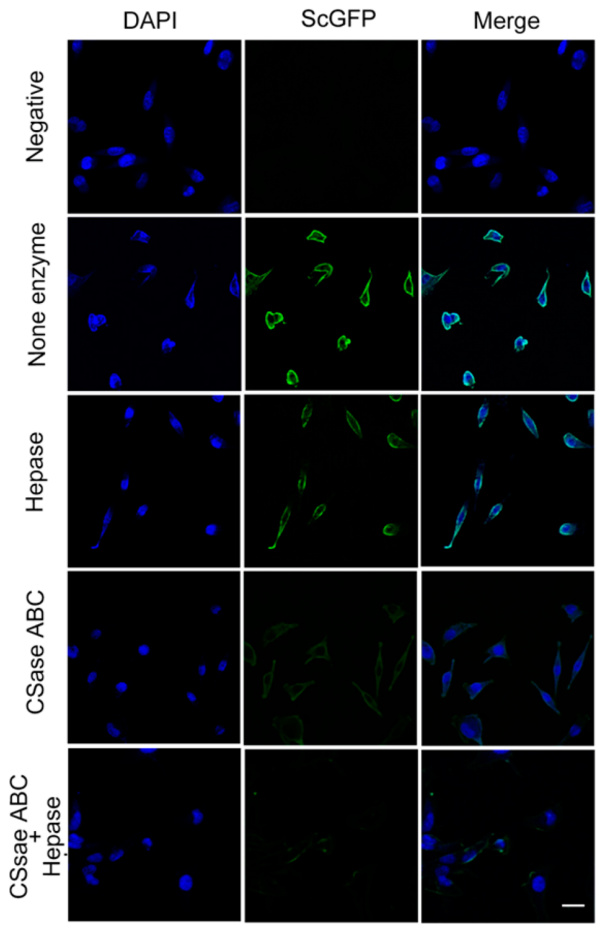


Figure 3. Visualization of GAGs on the surface of living cells. A549 cells cultured on a dish with a glass bottom were treated with or without the indicated enzymes and were further stained with ScGFP (green). The nucleus was stained with DAPI (blue). Scale bars: 20 μm.

Furthermore, the staining of A549 cells could be partially removed by treatment with CSase ABC, which specifically cleaves CS/DS. This suggests that the CS/DS chains of cell membrane PGs were involved in the interaction with ScGFP. A similar phenomenon was found in the treatment of cells with Hepases, which specifically digests HS/Hep. This suggests the involvement of cell surface HS in the interaction with ScGFP too. The cell surface staining with ScGFP could be almost completely abolished by digestion with CSase ABC and Hepases together, suggesting that cell surface GAGs, in particular CS and HS chains, were the predominant acceptors for the ScGFP. In addition, the results show that treatment with CSase ABC removes more ScGFP staining than treatment with Hepases, indicating that A549 cells express more CS/DS than HS on the cell surface. This finding is consistent with the results from the GAG disaccharide composition assay by HPLC (Figure S11 and Table S2).

The staining of the cell surface GAGs by ScGFP can be easily quantified by fluorescence-activated cell sorting (FACS). As shown in Figure S12, 293T cells can be stained by ScGFP with a high fluorescence intensity of 1901 (Figure S12A), and the majority (74%) of the fluorescence intensity can be reduced by treatment with both CSase ABC and Hepases (Figure S12D). In the case of 293T cells, treatment with CSase ABC (Figure S12B) removes less of the fluorescence intensity than that with Hepases (Figure S12C), suggesting that 293T cells express more HS than CS on the cell surface. This finding is consistent with the results from the GAG disaccharide composition assay by HPLC (Figure S11 and Table S2).

Further, to investigate whether ScGFP can be used as a probe to detect the distribution of GAGs *in vivo*, ScGFP was injected into mice through the tail veins. In a time course assay, we could see that ScGFP was mainly detained in the liver, stomach, kidney, and intestine. The accumulation of ScGFP in these organs reached the highest level 30 min after injection and then gradually decreased as time went on (Figure S13). Remarkably, the organ staining was still significant 8 h after injection, indicating that the cleanup of ScGFP *in vivo* is relatively slow. In fact, when we injected the starting GFP (StGFP) with a net charge of −7, the staining was detected only in the kidney after 30 min, indicating that the StGFP was quickly cleaned up from the blood (Figure S14). To demonstrate that the selective organ staining was due to the binding of ScGFP to GAGs, GAG-degrading enzymes were intravenously preinjected into the mice 1 h before ScGFP injection. As shown in Figure 4, organ staining can be completely abolished by pretreatment with both CSase ABC and Hepases, suggesting that the retention of ScGFP is due to the GAGs expressed in these organs. Furthermore, we noticed that the effect of treatment with CSase ABC was much stronger than that with Hepases, indicating that CS/DS had a more important role than HS/Hep in the binding of ScGFP to organs. These new findings will not only deepen our understanding of the physiological function of GAGs but also show a possibility for delivering therapy reagents to GAG-overexpressed organs by supercharged proteins.

CONCLUSION

In conclusion, all results from this study show that ScGFP is a potential versatile and biocompatible probe for the visualization and detection of GAGs in complex biological samples from the molecular level to the cellular level and even to the entire body. Through a combination with GAG/PG-specific enzymes and

DOI: 10.1021/acs.analchem.5b02071
Anal. Chem. 2015, 87, 9302−9307

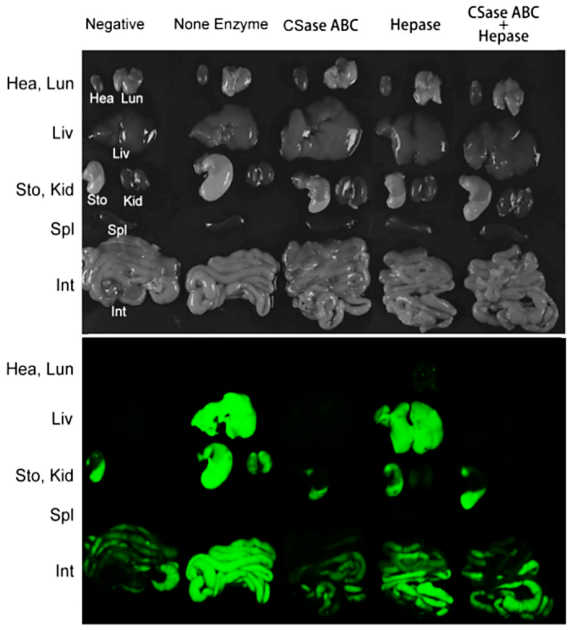


Figure 4. Visualization of GAGs in organs. PBS, CSase ABC, Hepases, and both CSase ABC and Hepases were injected into mice via the tail vein. After 1 h, ScGFP was intravenously injected into the mouse, and the organs of each mouse were photographed after 30 min. Top: under visible light; bottom: under fluorescence light. Organs are indicated by their abbreviations: Hea, heart; Lun, lung; Liv, liver; Sto, stomach; Kid, kidneys; Spl, spleen; Int, intestine.

antibodies and with various new biosensor materials such as GO and gold nanoparticles, we can develop various useful ScGFP-based analysis platforms for studies of GAG-related research and applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b02071.

Experimental details and additional data (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Phone: +86-531-88365165. Fax: +86-531-88363002. E-mail: fuchuanli@sdu.edu.cn.

*Phone: +86-532-84022570. Fax: +86-532-84022570. E-mail: zhangxr7407@126.com.

Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by the Major State Basic Research Development Program of China (No. 2012CB822102), the National Natural Science Foundation of China (No. 21275003), the Shandong Province Science and Technology Development Plan (No. 2013GSF12106), and the Independent Innovation Plan of Colleges and Universities in Jinan (No. 201401242).

■ REFERENCES

(1) Mizumoto, S.; Sugahara, K. *FEBS J.* **2013**, *280*, 2462−2470.

(2) Sugahara, K.; Mikami, T. *Curr. Opin. Struct. Biol.* **2007**, *17*, 536−545.

(3) Sugahara, K.; Mikami, T.; Uyama, T.; Mizuguchi, S.; Nomura, K.; Kitagawa, H. *Curr. Opin. Struct. Biol.* **2003**, *13*, 612−620.

(4) Sugahara, K.; Kitagawa, H. *Curr. Opin. Struct. Biol.* **2000**, *10*, 518−527.

(5) Laremore, T. N.; Zhang, F.; Dordick, J. S.; Liu, J.; Linhardt, R. J. *Curr. Opin. Chem. Biol.* **2009**, *13*, 633−640.

(6) Capila, I.; Linhardt, R. J. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 391−412.

(7) Linhardt, R. J. *Chem. Biol.* **2004**, *11*, 420−422.

(8) Yip, G. W.; Smollich, M.; Goette, M. *Mol. Cancer Ther.* **2006**, *5*, 2139−2148.

(9) Silbert, J. E.; Sugumaran, G. *IUBMB Life* **2002**, *54*, 177−186.

(10) Wright, A. T.; Zhong, Z. L.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2005**, *44*, 5679−5682.

(11) Mecca, T.; Consoli, G. M. L.; Geraci, C.; La Spina, R.; Cunsolo, F. *Org. Biomol. Chem.* **2006**, *4*, 3763−3768.

(12) Briza, T.; Kejik, Z.; Cisarova, I.; Kralova, J.; Martasek, P.; Kral, V. *Chem. Commun.* **2008**, 1901−1903.

(13) Sauceda, J. C.; Duke, R. M.; Nitz, M. *ChemBioChem* **2007**, *8*, 391−394.

(14) Egawa, Y.; Hayashida, R.; Seki, T.; Anzai, J. *Talanta* **2008**, *76*, 736−741.

(15) Zhong, Z. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2002**, *124*, 9014−9015.

(16) Lawrence, M. S.; Phillips, K. J.; Liu, D. R. *J. Am. Chem. Soc.* **2007**, *129*, 10110−10112.

(17) McNaughton, B. R.; Cronican, J. J.; Thompson, D. B.; Liu, D. R. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 6111−6116.

(18) Cronican, J. J.; Thompson, D. B.; Beier, K. T.; McNaughton, B. R.; Cepko, C. L.; Liu, D. R. *ACS Chem. Biol.* **2010**, *5*, 747−752.

(19) Lei, C.; Huang, Y.; Nie, Z.; Hu, J.; Li, L.; Lu, G.; Han, Y.; Yao, S. *Angew. Chem., Int. Ed.* **2014**, *53*, 8358−8362.

(20) Wang, Z.; Li, Y.; Li, L.; Li, D.; Huang, Y.; Nie, Z.; Yao, S. *Chem. Commun.* **2015**, *51*, 13373−13376.

(21) Tang, S.; Nie, Z.; Li, W.; Li, D.; Huang, Y.; Yao, S. *Chem. Commun.* **2015**, DOI: 10.1039/C5CC04170H.

(22) Guo, Y. N.; Lu, X.; Weng, J.; Leng, Y. *J. Phys. Chem. C* **2013**, *117*, 5708−5717.

(23) Wei, X. Q.; Hao, L. Y.; Shao, X. R.; Zhang, Q.; Jia, X. Q.; Zhang, Z. R.; Lin, Y. F.; Peng, Q. *ACS Appl. Mater. Interfaces* **2015**, *7*, 13367−13374.

(24) Li, S.; Mulloor, J. J.; Wang, L.; Ji, Y.; Mulloor, C. J.; Micic, M.; Orbulescu, J.; Leblanc, R. M. *ACS Appl. Mater. Interfaces* **2014**, *6*, 5704−5712.

(25) Hileman, R. E.; Fromm, J. R.; Weiler, J. M.; Linhardt, R. J. *BioEssays* **1998**, *20*, 156−167.

(26) Kim, D. H.; Park, Y. J.; Jung, K. H.; Lee, K. H. *Anal. Chem.* **2014**, *86*, 6580−6586.

(27) Cao, Y.; Shi, S.; Wang, L.; Yao, J.; Yao, T. *Biosens. Bioelectron.* **2014**, *55*, 174−179.

(28) Liu, J. M.; Chen, J. T.; Yan, X. P. *Anal. Chem.* **2013**, *85*, 3238−3245.

(29) Qi, H.; Zhang, L.; Yang, L.; Yu, P.; Mao, L. *Anal. Chem.* **2013**, *85*, 3439−3445.

(30) Wang, X.; Chen, L.; Fu, X.; Chen, L.; Ding, Y. *ACS Appl. Mater. Interfaces* **2013**, *5*, 11059−11065.

(31) Ling, Y.; Gao, Z. F.; Zhou, Q.; Li, N. B.; Luo, H. Q. *Anal. Chem.* **2015**, *87*, 1575−1581.

(32) Dai, Q.; Liu, W.; Zhuang, X.; Wu, J.; Zhang, H.; Wang, P. *Anal. Chem.* **2011**, *83*, 6559−6564.

(33) Guerrini, M.; Beccati, D.; Shriver, Z.; Naggi, A.; Viswanathan, K.; Bisio, A.; Capila, I.; Lansing, J. C.; Guglieri, S.; Fraser, B.; Al-

DOI: 10.1021/acs.analchem.5b02071
Anal. Chem. 2015, 87, 9302−9307

Hakim, A.; Gunay, N. S.; Zhang, Z.; Robinson, L.; Buhse, L.; Nasr, M.; Woodcock, J.; Langer, R.; Venkataraman, G.; Linhardt, R. J.; Casu, B.; Torri, G.; Sasisekharan, R. *Nat. Biotechnol.* **2008**, *26*, 669−675.
(34) Kishimoto, T. K.; Viswanathan, K.; Ganguly, T.; Elankumaran, S.; Smith, S.; Pelzer, K.; Lansing, J. C.; Sriranganathan, N.; Zhao, G.; Galcheva-Gargova, Z.; Al-Hakim, A.; Bailey, G. S.; Fraser, B.; Roy, S.; Rogers-Cotrone, T.; Buhse, L.; Whary, M.; Fox, J.; Nasr, M.; Dal Pan, G. J.; Shriver, Z.; Langer, R. S.; Venkataraman, G.; Austen, K. F.; Woodcock, J.; Sasisekharan, R. *N. Engl. J. Med.* **2008**, *358*, 2457−2467.
(35) Pan, J.; Qian, Y.; Zhou, X.; Pazandak, A.; Frazier, S. B.; Weiser, P.; Lu, H.; Zhang, L. *Nat. Biotechnol.* **2010**, *28*, 203−207.
(36) Sommers, C. D.; Mans, D. J.; Mecker, L. C.; Keire, D. A. *Anal. Chem.* **2011**, *83*, 3422−3430.
(37) Aich, U.; Shriver, Z.; Tharakaraman, K.; Raman, R.; Sasisekharan, R. *Anal. Chem.* **2011**, *83*, 7815−7822.
(38) Li, G.; Cai, C.; Li, L.; Fu, L.; Chang, Y.; Zhang, F.; Toida, T.; Xue, C.; Linhardt, R. J. *Anal. Chem.* **2014**, *86*, 326−330.
(39) Wang, L.; Buchanan, S.; Meyerhoff, M. E. *Anal. Chem.* **2008**, *80*, 9845−9847.
(40) Sommers, C. D.; Keire, D. A. *Anal. Chem.* **2011**, *83*, 7102−7108.
(41) Kalita, M.; Balivada, S.; Swarup, V. P.; Mencio, C.; Raman, K.; Desai, U. R.; Troyer, D.; Kuberan, B. *J. Am. Chem. Soc.* **2014**, *136*, 554−557.
(42) Luehn, S.; Schrader, T.; Sun, W.; Alban, S. *J. Pharm. Biomed. Anal.* **2010**, *52*, 1−8.

DOI: 10.1021/acs.analchem.5b02071
Anal. Chem. 2015, 87, 9302−9307