PHOTOSENSITIVE HEPARINS

MARTA STOLAREK*, KRZYSZTOF SZCZUBIAŁKA

FACULTY OF CHEMISTRY, JAGIELLONIAN UNIVERSITY, POLAND
*E-MAIL: MARTA.STOLAREK@DOCTORAL.UJ.EDU.PL

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Introduction

Heparin is a polysaccharide known for its important role in human physiology, mostly for its influence on blood coagulation and interaction with heparin-binding proteins, e.g., growth factors (FGF, VEGF) [1, 2]. In its native form heparin, like other polysaccharides, does not absorb light from the near ultraviolet/visible range of the spectrum, thus its biological activity is not responsive to light. Being a polysaccharide, heparin can be easily functionalized. Polysaccharides undergo facile esterification, etherification, and oxidation reactions [3]. Polysaccharides containing cis-trans photoisomerizable chromophores were also obtained. It was found that photoisomerization of these chromophores significantly changed the properties of the polysaccharides [4].
Therefore, it is postulated that functionalization of heparin with a photoisomerizable compound such as the arylazopyrazole (AAP)-type photoswitch, either by substitution or grafting, will allow gaining photocontrol over physicochemical properties of heparin (e.g., hydrophilicity/hydrophobicity, viscosity, solubility, chain conformation, interactions with other (macro)molecules), and, consequently, its biological activity.

Materials and Methods

Synthesis and characterization of photoactive UFH heparin

Since unfractionated heparin is scarcely soluble in organic solvents, it (Heparinum WZF, Polfa) was first converted into the ammonium salt soluble in DMF by reacting it with Hyamine 1622, using the method described in the literature [5]. In the heparin hyamate obtained the carboxyl groups are neutralized with sodium cations and the sulfate groups are neutralized with hyamine ammonium cations.
Then, the obtained salt was passed through a carboxyl ion-exchange Bio-rex 70 resin (in the acidic form) to obtain heparin form in which the sulfate groups were salified with hyamine 1622 and the carboxyl groups were in the reactive acidic form. The photoactive heparin derivatives were obtained via esterification of the carboxyl groups with AAP-type photoswitch. The esterification was performed using a method described in the literature [6]. For quantitative photoisomerization of the photoswitch attached to heparin 365 nm (trans-cis) and 530 nm (cis-trans) light was used. The half-life time of the cis isomer formed was checked by UV-Vis measurements. The influence of trans-cis isomerization on the hydrodynamic diameter was investigated by DLS method.

Results and Discussion

The applied synthesis pathway allowed the attachment of a photoswitch to unfractionated heparin (UFH). Depending on the ratio of the molar reactants, different degrees of substitution could be obtained. The trans-cis isomerization under 365 nm irradiation (FIG. 1) is quick (about 1 minute under LED irradiation), while the reverse process (cis-trans) under 530 nm irradiation was much longer (about 1 hour). DLS investigations of obtained derivatives of heparins showed that the trans-cis photoisomerization reduces the hydrodynamic diameter and this effect was more pronounced for higher degrees of UFH substitution. Studies of the cis isomer stability at different temperatures showed that the thermal half-life of the cis isomer in the physiological temperature is exceptionally long, opening possible biomedical applications for the photosensitive heparin.

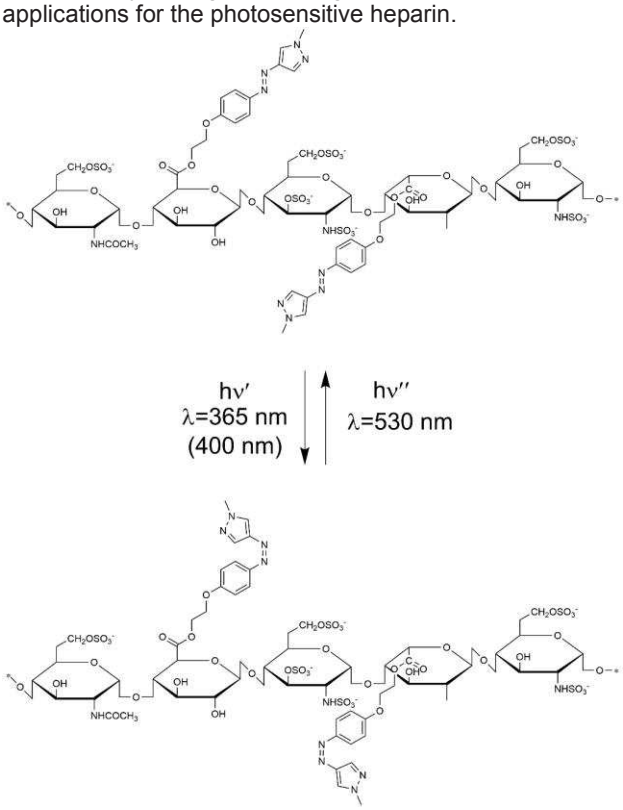

FIG. 1. Photoisomerizations in UFH heparin substituted with an AAP-type photoswitch.

Conclusions

Photosensitive heparins were obtained. Trans-cis and cis-trans isomerizations occur under 365 nm and 530 nm irradiation, respectively. The photoisomerization process influences the hydrodynamic diameter of UFH. Obtained photocontrollable systems are characterised by unusually long thermal half-life of the cis isomer.

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References

[1] L. D. Thompson, et al., Biochemistry. 33, (2002) 831–3840.
[2] S. N. Bolten, et al., Appl. Microbiol. Biotechnol. 102, (2018) 8647–8660.
[3] A. Kirschning, et al., A Eur. J. 24, (2018) 1231–1240.
[4] H. Wondraczek, et al., Carbohydr. Polym. 83, (2011) 1048– 1061.
[5] G. Nominé, et al., US2989438A patent (1961).
[6] J. Mardiguian, et al., US3891622 patent (1975).