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Design and synthesis of structurally defined heparan sulfate (HS)-FK506 conjugates as an exogenous approach to investigate biological functions of nucleus HS

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ABSTRACT

Although heparan sulfate (HS) is widely implicated in numerous physiological and pathological processes, the biological function of nucleus HS remains underexplored, largely due to its complex structure and high hydrophilic property. To supplement these efforts, ideal vehicles are drawing attention as they combine attractive features including lipid solubility for penetrating cell membrane, high affinity binding to its target receptor, metabolic stability, and no cellular actions resulting in toxicity. Herein, we develop a convenient and promising strategy to prepare HS-FK506 conjugates for membrane transport and entry into nucleus, where click chemistry takes easily place between the exocyclic allyl group of a clinic drug FK506 and thiol as a handle incorporated into HS analogues. HS derivatives for constructing the conjugates were synthesized using a cutting-edge chemoenzymatic method. Meantime, [^{35}S] labeled 3′-phosphoadenosine 5′-phosphosulfate (PAP^{35}S) and [^{14}C] glucuronic acid (Glc A) were adopted to label HS-FK506 conjugates, respectively, to evaluate their efficiency of nucleus entry, as a result, ^{14}C Glc A was sensitive, effective and reliable whereas PAP^{35}S gave rise to a mixture of labeled compounds, hampering the understanding of structure-function relationship of nucleus HS. Compared with the corresponding HS, the amount of HS-FK506 conjugates to translocate into nucleus from radioactive assay experiments sharply increased, e.g. tridecasaccharide-FK506 **1d** increased by approximate 10 folds, offering a simple and robust platform for enabling hydrophilic compounds including carbohydrates to translocate into nucleus and shedding light on their biological functions.

1. Introduction

Heparan sulfate (HS) is a sulfated and linear biopolymer, carrying the disaccharide-repeating units composed of alternating glucuronic acid (Glc A) or iduronic acid (Ido A) and glucosamine residues. HS, which is assembled in the Golgi apparatus and belongs to the family of glycosaminoglycans (GAGs), usually participates in multiple physiological and pathological processes at cell-tissue-organ interface, including controlling blood coagulation [1], assisting viral and bacterial infections [2] and regulating tumor growth, metastasis and angiogenesis [3], etc. To accommodate to the requirements of varied biological activities, considerable structural complexities of HS, containing diverse substitution with *O*-sulfo, *N*-acetyl, and *N*-sulfo groups, are dominated by a series of enzyme-processing reactions in a rigorous sequential manner. Analyses of HS isolated from different mammalian tissues are in support of tissue-specific HS. Moreover, any subtle alterations in HS expression are found to be closely associated with many diseases [3].

Although numerous compelling evidences support the importance of HS, resided on the cell surface and in the extracellular matrix (ECM), in mediating the biological activities, the bio-functions of nucleus HS lay far behind, leading to the substantially underestimated consequences. HS was first reported in the nucleus of hepatocytes in 1986[2], and a mounting number of nucleus HS derivatives have been found to be likely more prevalent than we considered under the help of high resolution imaging and high affinity antibodies. Similarly, nucleus HS exists in the diversified structural formation as one in ECM, encoding different information to control its potential cellular behaviors, such as the cell cycle, proliferation and transcription etc [4,5].

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However, it is unclear how nucleus HS exactly mediates those cellular behaviors and further performs the corresponding biology. More disappointingly, lack of structure-activity relationship (SAR) of nucleus HS-mediating effects makes it difficult to understand the physiology and pathology. Therefore, it is urgent to develop a potential approach to dissect the SAR of nucleus HS and deeply probe into its molecular mechanism.

Inspired by a model derived from the previous studies on ECM HS, where HS structural diversity is not random, but rigorously regulated in a context-dependent manner [6,7], it is possible that to artificially manoeuvre specific nucleus HS can fine-tune corresponding physiological or disease processes. The introduction of exogenously diversified HS into nucleus can controllably interfere with nucleus HS function, probably providing the experimental basis for identifying the function of nucleus HS and understanding its SAR.

The choice of an external molecular cargo that can transport exogenous HS into cell nucleus may be of paramount importance. Theoretically, ideal characteristics for a cargo that is applied to shuttle or carry exogenous substance into cell nucleus, are endowed with lipid solubility leading to penetrating cell membrane, high affinity binding to its target receptor, metabolic stability, and no cellular actions resulting in toxicity. The FK506 (tacrolimus) molecule is regarded as a desired precursor to such a molecule [8]. It readily crosses cell membranes and specifically binds to FKBP12 thought to participate in intracellular transport similar to the chaperones [8,9], holding great potentials in carrying exogenous functional molecules. FK506 is clinically an immunosuppressant drug with the privileged structure macrolide, therefore, the conversion of FK506 into non-immunosuppressive and nontoxic matchmaker is crucial. With the careful analysis of SAR and immunosuppressive mechanism of FK506, exocyclic allyl moiety of FK506 is an important element for calcieurin-binding to trigger immunosuppression effects [9]. Tethering allyl substituent with the appropriate moiety to block the ability of immunosuppression, FK506 can be converted into a non-immunosuppressive and nontoxic carrier. As a result, exocyclic allyl group on FK506 may be a potential modifying site to carry functional molecule HS into cells or even nucleus.

To address the feasibility for dissecting the biological functions of nucleus HS and exploring SAR, as seen in Fig. 1, the incorporation of HS to exocyclic allyl group on FK506 with a suitable linkage could represent a novel approach to translocate HS into cell nucleus. Specifically speaking, the exogenously diversified HS derivatives were transported into cell nucleus in the formation of FK506-HS conjugates, and they was coupled with a thioether linkage acquired by the thiol-ene photoreaction [10]. HS derivatives used for developing FK506-HS conjugates, were chemo- enzymatically prepared. Meantime, Two radiolabeled compounds, [^{14}C] Glc A, starting from commercially available [^{14}C] glucose, and PAP^{35}S prepared as the procedure proposed previously [11], were applied to be attached to HS to monitor FK506-HS respectively. In this framework, four typical HS analogues (**5a-d**) with different sizes and substituent modes were selected to conjugate with FK506, and the corresponding [^{14}C] HS-FK506 derivatives labeled at their non-reducing end with UDP-[^{14}C] GlcA were also synthesized, for the purpose of assessing the abilities of nucleus entry with radioactive assay experiments.

2. Results and discussion

One huge challenge in HS studies is to make HS oligosaccharides widely available to the scientific research community. Although HS oligosaccharides can be synthesized by purely chemical approaches, the synthetic routes are long and costly, and are unsuitable for preparing HS oligosaccharides larger than hexasaccharides [12]. Employing a modular synthetic approach, selective sulfation of hydroxyls and amines of partially protected oligosaccharides should give access to many compounds for conjugates development [13], but such a strategy also requires a relatively large number of chemical steps for theirs preparation, leading to the high-risk synthesis of several different sulfates target derivatives. As such, a chemoenzymatic approach is drawing our attentions that can synthesize heparin and HS with high efficiency and high yield [6,7]. The chemoenzymatic method that is by far the most cost-effective approach to supply the HS oligosaccharides has been steadily improved in terms of synthesis scale and product purity [14]

To simplify the synthetic process but exhibit the structural

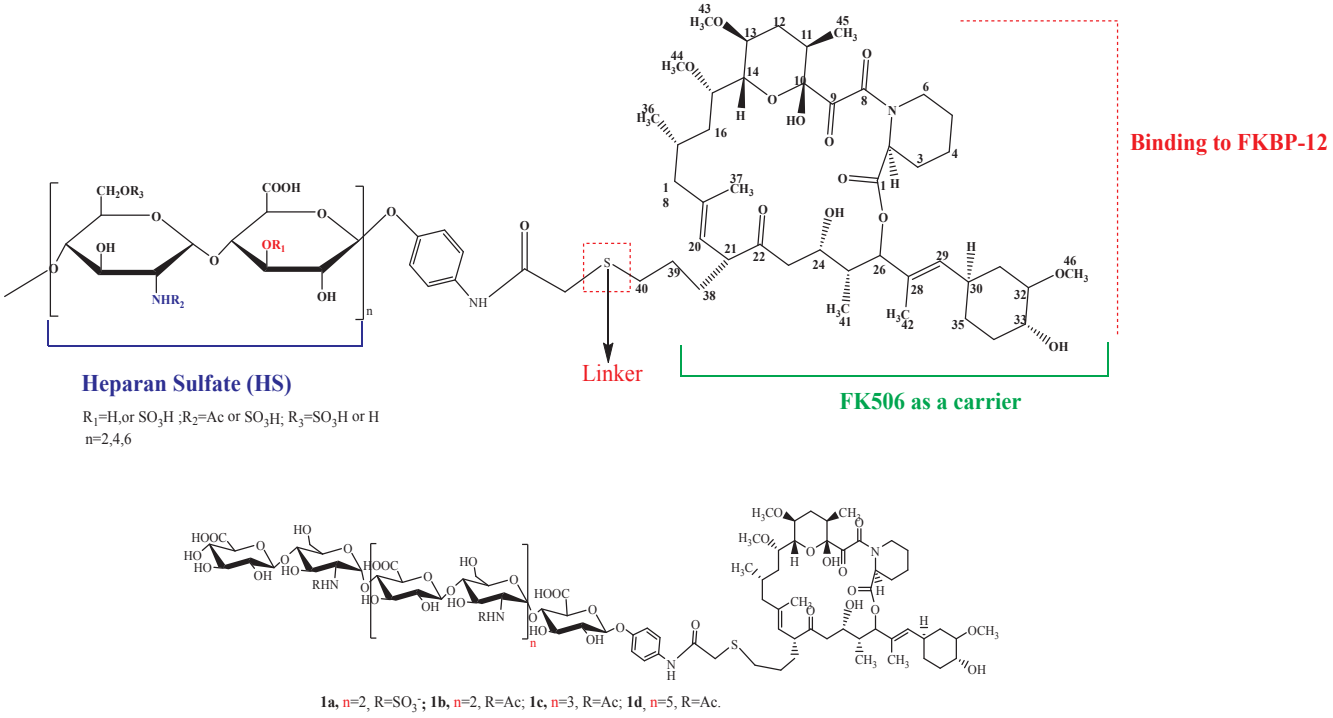

Fig. 1. The general structure of HS-FK506 conjugates designed and detailed structures of compounds **1a-d**.