Light-induced 3-O-Sulfotransferase Expression Alters Pineal Heparan Sulfate Fine Structure

A SURPRISING LINK TO CIRCADIAN RHYTHM*

Received for publication, November 13, 2003
Published, JBC Papers in Press, November 20, 2003, DOI 10.1074/jbc.C300492200

Balagurunathan Kuberan, Miroslaw Lech, Jimmy Borjigin, and Robert D. Rosenberg

From the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, the Division of Molecular and Vascular Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215 and the Department of Molecular and Integrative Physiology, University of Michigan School of Medicine, Ann Arbor, Michigan 48109

Proteoglycans are dominant glycoconjugates located on the cell surface and in extracellular spaces and consist of a core protein with one or more glycosaminoglycan side chains linked covalently. Heparan sulfate (HS) belongs to the family of glycosaminoglycans. HS has been assigned a variety of physiological and pathological functions, such as cell-cell adhesion, cell-matrix adhesion, cell proliferation, motility and differentiation, lipoprotein metabolism, blood coagulation, inflammation, tissue regeneration, tumor progression and invasion, pathogenic infection by bacteria, protozoa, and viruses, through specific interaction with a wide array of proteins, ligands, receptors, and pathogens (Bernfield, M., Gotte, M., Park, P. W., Reizes, O., Fitzgerald, M. L., Lincecum, J., and Zako, M. (1999) Annu. Rev. Biochem. 68, 729–777). We have shown here for the first time that light induces changes in pineal HS fine structure and that occurrence of the rare 3-O sulfation catalyzed by HS 3-sulfotransferase (3-OST2) is predominantly restricted to daytime pineal glands.

HS is ubiquitously expressed on the cell surface and has been shown to regulate many different biological functions (1). It is a highly acidic polysaccharide with repeating disaccharide units consisting of a glucosamine and hexuronic acid (ido- and/or gluc-). HS is biosynthesized in the Golgi by the addition of nucleotide sugars to the reducing end of the growing polysaccharide chain followed by subsequent modification by different enzymes in a concerted fashion (2). The nascent chain may be epimerized at the C-5 position and/or sulfated at the C-2 position of uronic acid residues, and may be N- or O-sulfated and/or N-acetylated glucosamine residues. Although core proteins have fairly homogeneous compositions, the lengths and compositions of HS chains are highly variable (2). Heparan sulfate glucosaminyl 3-O-sulfotransferase (3-OST) was the first biosynthetic enzyme found to be present in multiple isoforms and carry out precursor structure specific rare 3-O modification (3). HS glucosaminyl 3-OST1 isoform was demonstrated to be important for the anticoagulant function of HS in which anti-thrombin III specifically binds to 3-OST1 modified HS structure, whereas 3-OST3a isoform is critical for entry of herpes simplex virus into the cell where the interaction of viral gD protein with 3-OST3a-modified HS occurs (4–6). 3-OST2 and 3-OST4 isoforms were found to be exclusively and abundantly expressed in many different areas of the brain. However, knowledge of their functions remains elusive.

The pineal gland is a neuroendocrine organ of the brain that synthesizes and secretes melatonin, a nocturnal hormone implicated in the reproductive functions of seasonal animals, clock resetting, and sleep modulation. By differential analysis of pineal day and night mRNA, we have identified a large amount of 3-OST2 in the pineal gland in which expression is restricted to daytime (7). However, it was not known whether the increase in 3-OST2 mRNA levels would lead to an altered HS fine structure. On the one hand, immunological reagents to specifically detect 3-OST2 are not available. On the other hand, the presence of an appropriate HS precursor structure in pineal glands for modification by 3-OST2 is unknown. For these reasons, we investigated whether light induces changes in pineal HS fine structure because of a rare 3-O modification. This may provide novel molecular insights into the link between heparan sulfate glycochemistry and the largely elusive circadian biology.

EXPERIMENTAL PROCEDURES

Materials—Heparitinase I, II, and III derived from Flavobacterium hirpinutioni were purchased from Seikagaku USA (Cape Cod, MA). All chemicals and LC/MS grade solvents were from either Sigma or Aldrich. DEAE-Sephacel material was purchased from Amersham Biosciences. Adult male Sprague-Dawley rats purchased from Harlan Sprague-Dawley were housed in a temperature-controlled room under 14:10 light/dark conditions with lights-off at 1 a.m. for more than 1 week before experiments. Day pineals were harvested at 2 p.m., and the night pineals were isolated at 7 a.m. under red light and immediately placed in dry ice.

Isolation of Pineal Heparan Sulfate—Pineals were homogenized and treated with Pronase solution (150 μg of protease type XIV from Streptomyces griseus in 1 ml of 40 mM NaOAc, 320 mM NaCl, pH 6.5). Proteolysis was carried out at 37 °C for 24 h. The digestion mixture was then purified as described earlier to isolate heparan sulfate for subsequent LC/MS characterization (8).

Enzymatic Digestion of Day and Night Pineal Heparan Sulfate Polysaccharides—HS isolated from five day and five night pineals was digested with 0.33 milliunits of heparitinase I, II, and III in 50 μl of 40 mM ammonium acetate buffer (pH 7.0) containing 3.3 mM CaCl2 at 22 °C for 24 h. Then the reaction mixture was taken out and boiled at 100 °C for 2 min to inactivate the enzyme, and a fraction of reaction mixture (10 μl) was directly loaded to capillary HPLC for LC/MS analysis as we described previously (8).

RESULTS AND DISCUSSION

Previously we demonstrated that 3-OST2 expression is abundant during the daytime and is light-inducible at night through the suppression of β-adrenergic signaling (7). Despite this finding, it was unclear whether induced 3-OST2 RNA
disaccharides were resolved to homogeneity by capillary HPLC and were then analyzed by on-line coupled electrospray ionization mass spectrometry. The observed m/z of 785 corresponds to a tetrasulfated disaccharide molecular ion ([M – 2H + IDBA]−, Fig. 1 (Day Pineal), and thus the structure is Δ U2S-GlcNS3S6S. The mass spectrum clearly shows the differences in the 3-O-sulfated disaccharides derived from the digestion of day and night pineal HS polysaccharides. This difference corroborates the nature of the modification controlled by day and night cycle. It seems probable that this light-induced modified HS interacts with a specific protein within or outside of the pineal and may be a part of the molecular machinery that is responsible for circadian rhythm. We are currently attempting to identify this putative downstream target by a combination of proteomic and synthetic heparan sulfonate approaches (10–12). This is likely to represent a novel pathway not involving melatonin as suggested by Borjigin et al. (7). We are also currently attempting to understand the mechanism by which light influences the transcription of the pineal 3-OST2. As light acts indirectly by termination of the adrenergic signaling to the pineal gland, and β-adrenergic (7) and cAMP signaling (8) suppress the 3-OST2 transcription, we hypothesize that a cAMP response element is present in the 3-OST2 promoter and that the transcriptional activation of the 3-OST2 gene requires the silencing of the cAMP response element. We have previously shown that 3-OST2 enzyme preferentially sulfates glucosamine residues that are located to the reducing side of 2-O-sulfated glucuronic acid, which is known to be present in various areas of the brain (13). In the present study, we utilized heparin lyases to cleave the minute quantity of pineal HS into disaccharides for the structural analysis, although with the loss of stereochemical information of uronic acids. In order to determine unequivocally the structures in the vicinity of the 3-O-sulfated glucosamine residues, it is essential to utilize other cleavage techniques that are unfortunately not compatible with mass spectrometry at the present time. We surmise that the pineal HS fine structure contains these two unusual residues that may specifically interact with a protein ligand to regulate a hitherto unknown pathway involved in circadian biology. In summary, this communication for the first time demonstrates the involvement of an HS fine structure in circadian rhythm. It is likely that other neurobiological functions may also be subserved by this brain-specific sulfotransferase (3, 4).

REFERENCES
1. Bernfield, M., Gotti, M., Park, P. W., Reizes, O., Fitzgerald, M. L., Linecuezum, J., and Zako, M. (1999) J. Biol. Chem. 274, 728–737.
2. Rosenberg, R. D., Shworak, N. W., Liu, J., Schwartz, J. J., and Zhang, L. J. (1997) J. Clin. Investig. 99, 2062–2070.
3. Shworak, N. W., Liu, J. A., Peterson, L. M., Zhang, L. J., Kobaryashi, M., Copeland, N. G., Jenkins, N. A., and Rosenberg, R. D. (1999) J. Biol. Chem. 274, 5170–5184.
4. Shworak, N. W., Liu, J., Fritze, L. M. S., Schwartz, J. J., Zhang, L. J., Logeart, D., and Rosenberg, R. D. (1997) J. Biol. Chem. 272, 28008–28019.
5. Liu, J. A., Shworak, N. W., Sinay, P., Schwartz, J. J., Zhang, L. J., Fritze, L. M. S., and Rosenberg, R. D. (1999) J. Biol. Chem. 274, 5185–5192.
6. Shukla, D., Liu, J., Blaklock, P., Shworak, N. W., Bai, X. M., Esko, J. D., Cohen, G. H., Eisenberg, R. J., Rosenberg, R. D., and Spear, P. G. (1999) Cell 99, 13–22.
7. Borjigin, J., Deng, J., Sun, X., De Jesus, M., Liu, T., and Wang, M. M. (2003) J. Biol. Chem. 278, 16315–16319.
8. Kuberan, B., Lech, M., Zhang, L., Wu, Z. L., Beeler, D. L., and Rosenberg, R. D. (2002) J. Am. Chem. Soc. 124, 6701–6718.
9. Zhang, L. J., Lawrence, R., Schwartz, J. J., Bai, X. M., Wei, G., Esko, J. D., and Rosenberg, R. D. (2001) J. Biol. Chem. 276, 28866–28873.
10. Kuberan, B., Beeler, D. L., Lawrence, R., Lech, M., and Rosenberg, R. D. (2003) J. Am. Chem. Soc. 125, 12424–12425.
11. Kuberan, B., Lech, M. Z., Beeler, D. L., Wu, Z. L., and Rosenberg, R. D. (2003) Nat. Biotechnol. 21, 1343–1346.
12. Kuberan, B., Beeler, D. L., Lech, M., Wu, Z. L., and Rosenberg, R. D. (2003) J. Am. Chem. Soc. 125, 52913–52921.
13. Rong, J., Habuchi, H., Kimata, K., Lindahl, U., and Kusche-Gullberg, M. (2001) Biochemistry 40, 5548–5555.

Fig. 1. Mass spectra of heparitinase-treated HS isolated from night (upper panel) and day (lower panel) pineal glands.