Proteoglycan Degradation

Cleavage of aggrecan within the interglobular domain (IGD) at the Glu373-Ala374 bond has been attributed to the protease "aggrecanase" (1,2). In addition to this cleavage site, aggrecan has been shown to cleave within the C-terminal region of aggrecan between G2 and G3 (3). The core protein of aggrecan is highly glycosylated with regions rich in both keratan sulfate (KS) and chondroitin sulfate (CS) (4). Since age-related changes in aggrecan glycosylation have been observed (4), these glycosaminoglycans (GAGs) may play a role in controlling aggrecan degradation. We had previously shown that although fully glycosylated aggrecan is readily cleaved by aggrecanase at the Glu373-Ala374 bond, this cleavage is not observed when the aggrecan substrate is deglycosylated with chondroitinase ABC and keratanase. Studies by Barry et al. (4) have shown that a KS chain is present within the sequence that includes the Glu373-Ala374 cleavage site in bovine aggrecan. Thus, our data suggest that this KS chain may be important for recognition of this site by aggrecanase. Aggrecan, treated with chondroitinase ABC to remove CS, was cleaved by aggrecanase at the Glu373-Ala374 bond, although at a lower rate. Under these conditions, several larger MW fragments were produced by cleavage at the Glu373-Ala374 site, suggesting that loss of CS may be interfering with aggrecanase cleavage at sites within the C-terminal domain of aggrecan. To confirm this observation, we evaluated these samples for cleavage at the Glu1714-Gly1715 bond (GELE) and the Glu1545-Gly1546(KEEE) bond (KREEE) using neuropeptide antibodies to fragments generated by cleavage at these sites. Consistent with our observation that the aggrecan fragments produced by cleavage at the Glu1714-Gly1715 bond were of higher MW, fragments produced by cleavage at Glu1545-Gly1546 and Glu1545-Gly1546(KEEE) sites were not detected when aggrecan lacking CS was used as a substrate for aggrecanase. In contrast, removal of KS from aggrecan did not effect cleavage at these two sites. These data support our hypothesis that glycosylation of the aggrecan monomers affects recognition and cleavage by aggrecanase.

RESULTS AND DISCUSSION

Aggrecan digestion by aggrecanase results in several BC-3 reactive products, ranging in size from 98 kDa to 250 kDa. Prior treatment of the aggrecan substrate to remove both CS and KS chains resulted in complete inhibition of BC-3-reactive product generation, suggesting that these GAGs play a role in aggrecanase recognition of its substrate. In contrast, MMP-3 cleavage within the interglobular domain at the Asn341-Phe342 bond was not effected by deglycosylation of the aggrecan substrate. Cleavage at the Glu373-Ala374 bond was not detected when aggrecan, treated to remove KS, was used as a substrate for aggrecanase. However, a different pattern of cleavage products was detected by CSPG Western, suggesting that under these conditions, aggrecanase cleavage occurred at a different site (s). Studies by Barry et al. [4] have shown that a KS chain is present within the sequence that includes the Glu373-Ala374 cleavage site in bovine aggrecan. Thus, our data suggest that this KS chain may be important for recognition of this site by aggrecanase. Aggrecan, treated with chondroitinase ABC to remove CS, was cleaved by aggrecanase at the Glu373-Ala374 bond, although at a lower rate. Under these conditions, several larger MW fragments were produced by cleavage at the Glu373-Ala374 site, suggesting that loss of CS may be interfering with aggrecanase cleavage at sites within the C-terminal domain of aggrecan. To confirm this observation, we evaluated these samples for cleavage at the Glu1714-Gly1715 bond (GELE) and the Glu1545-Gly1546(KEEE) bond (KREEE) using neuropeptide antibodies to fragments generated by cleavage at these sites. Consistent with our observation that the aggrecan fragments produced by cleavage at the Glu373-Ala374 bond were of higher MW, fragments produced by cleavage at Glu1714-Gly1715 and Glu1545-Gly1546(KEEE) sites were not detected when aggrecan lacking CS was used as a substrate for aggrecanase. In contrast, removal of KS from aggrecan did not effect cleavage at these two sites. These data support our hypothesis that glycosylation of the aggrecan monomers affects recognition and cleavage by aggrecanase.

REFERENCES
