Plenary Lectures

CARTILAGE PROTEOGLYCANS: STRUCTURE, INTERACTIONS AND ORGANIZATION

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In hyaline cartilage a large proportion of proteoglycans are present as multimolecular aggregates in which many proteoglycans bind to single chains of hyaluronate.[1] Each proteoglycan has a complex structure in which many chondroitin sulphate and keratan sulphate chains are covalently attached by their reducing terminal ends to a polypeptide backbone. Structural studies on proteoglycans from bovine nasal cartilage have shown that the polypeptide backbone contains three distinct regions: (a) a region to which the majority of chondroitin sulphate chains and ∿ 20% of the keratan sulphate chains are attached; (b) a region to which more than half the keratan sulphate chains are attached; and, (c) a region, largely devoid of glycosaminoglycan chains, which contains a specific binding site for hyaluronate.[2,3] All proteoglycan preparations are polydisperse with variations in the number, type and length of glycosaminoglycan chains bound to each molecule and also in the length of the major chondroitin sulphate bearing region of the polypeptide.[3-6]

The formation of aggregates involves interactions between three components; proteoglycan, protein-link and hyaluronate, but only the interaction between proteoglycan and hyaluronate has been studied in any detail.[2,7-10] The binding site of the proteoglycan is in a globular region at one end of the proteoglycan protein core, and it has a high affinity for a decasaccharide unit of hyaluronate.[8,10] The binding activity of intact proteoglycan was studied after modification of cystine, lysine, arginine, tryptophan or tyrosine residues in the protein core; and, in each case, subsequent interaction with hyaluronate was blocked without evidence of peptide cleavage. The results showed that the binding activity was sensitive to alterations in the charge or hydrophobicity of residues which may be involved either in specific subsite interactions with hyaluronate or in maintaining the tertiary protein structure necessary for binding. However, with two reversible modifications, the reduction of disulphide bridges and the substitution of lysine groups with 2-methyl maleic anhydride, it was possible to regenerate binding activity.[5] This suggests that the native conformation of the binding site is thermodynamically preferred and has high inherent stability under the conditions of the experiment.

The specific protein link molecules involved in aggregation have been shown to occur in both a high molecular weight form (MW 45,000) and a low molecular weight form (MW 40,000) in bovine nasal cartilage.[11,12] Only the low molecular weight form is present in aggregates isolated from rat chondrosarcoma[13] while the high molecular weight form predominates in samples isolated from cultures of chick limb chondrocytes.[14] Experiments have suggested that the two forms are structurally related[15,16] with the larger containing a glycopeptide extension.[15] A comparison of the properties of proteoglycan aggregates formed with and without protein-link molecules showed that they functionally "locked" proteoglycans onto hyaluronate.[17] In contrast, the binding of proteoglycan to hyaluronate appeared to be an equilibrium. As a consequence, a higher concentration of hyaluronate (2% w/w relative to proteoglycan) was required to show satura-

tion binding in the analytical centrifuge than was needed with aggregates stabilized with protein-link (0.8% w/w hyaluronate under similar conditions).[18] The sedimentation coefficient of aggregates formed with hyaluronate fractions of different average size increased with the length of the hyaluronate chains and with their degree of saturation with proteoglycans. However, it appeared to be more dependent on the number of proteoglycans bound to each hyaluronate chain rather than on the length of the hyaluronate chain.[18] The sizes of proteoglycan aggregates are, thus, critically dependent on the molar ratio of proteoglycans to hyaluronate in the tissue.

Aggregation by interaction with hyaluronate appears to be specific to proteoglycans from cartilage and synthesis of proteoglycans based on a polypeptide with a hyaluronate binding site may thus be exclusive to chondrocytes. It is not yet clear whether other proteoglycans have specific properties that are characteristic of other types of cells. Although at least small amounts of proteoglycan are present in all tissues, most have not yet been subjected to the same detailed structural analysis as those from cartilage. It is, however, evident that the future classification of proteoglycans will depend heavily on the determination of the polypeptide structures of their core proteins.

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