ELECTRON MICROSCOPY OF ISOLATED PROTEOGLYCANS

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The structure and macromolecular organization of the proteoglycans (PG) of hyaline cartilage has to a large extent been clarified by the combined application of chemical and physical techniques. For a recent review, see ref. 1. However, the conclusions on shape, size and organization of these molecules can only be approximations due to the nature of the above methods. The application of the cytochrome c - spreading technique[2] to PG, making it possible to observe and measure individual isolated molecules in the electron microscope was therefore an important contribution[3]. Using this technique, Rosenberg *et al.*[3] could visualize the PG molecules as an extended central core filament to which side-chain filaments were attached at various intervals.

These observations were extended in a study[4,5] on PG monomers isolated from three different hyaline cartilages. The PG from the three sources were ultrastructurally rather similar. The distributions of core filament lengths ranged from about 170 to 370 nm in molecules from bovine nasal cartilage. Statistical evaluation revealed no significant deviations from a normal distribution in contrast to previous observations[3]. The use of high shear[3] and dissociative[4,5] preparation techniques, respectively, explains this difference. The observed average central filament length of some 300 nm is of the same magnitude as would be expected for a partially extended protein with a molecular weight (M_w) of 120,000, assuming a peptide length of 0.25 nm. The number of side-chain filaments was correlated to the length of the core filament, with an average of about 25. Since the number of chondroitin sulfate (CS) molecules in a PG monomer is about 100[1], it is likely that each side-chain filament observed represents a cluster of, on the average, 4 CS chains. The average lengths of the side-chain filaments in the three materials ranged from 39 to 46 nm. Assuming a disaccharide length of about 1 nm and a M_w of about 500 per disaccharide, this would correspond to a M_w of about 20,000 for the CS.

The above calculations agree with the current model for the PG monomer from hyaline cartilage[1]. It should be noted, however, that with the techniques used for visualization of monomers the terminal hyaluronic acid(HA)-binding region of the peptide core cannot be demonstrated. If the average molecular parameters found for monomers from bovine nasal cartilage (core length 290 nm, 25 side-chain filaments/core filament, length of side-chain filaments 45.6 nm) are used to calculate the M_w a value of 2.4×10^6 is obtained[6]. The range within the material of 100 measured molecules was $0.7 - 3.8 \times 10^6$. This value agrees well with the value of 2.3×10^6 calculated on the basis of chemical and physicochemical data[1]. Further statistical treatment of the ultrastructural data indicated that the average length of the side-chain filaments increased with increasing length of the protein core. If the hydrodynamic size of the CS-clusters is correlated to the length of the side-chain filaments this could indicate that the hydrodynamic size of the CS-clusters is smaller close to the HA-binding region[6].

The technique for visualizing PG monomers in the electron microscope was later modified to allow the observation of PG aggregates[7]. It was shown

that the aggregates consisted of PG monomers arising laterally at intervals of 20 to 30 nm from a filamentous backbone varying in length from 400 to 4,000 nm. It was suggested that the filamentous backbone corresponded to the HA of the PG aggregates. Moreover, the spacing of monomers corresponded to that suggested on the basis of chemical data[1].

In attempt to further investigate the molecular architechture of the PG aggregate native and fragmented aggregates were studied after spreading with cytochrome c [8,9]. Aggregates from bovine nasal cartilage were stepwise digested with chondroitinase and trypsin and the fragments purified and then observed in the electron microscope. HA was isolated from the same tissue after papain digestion. The general appearance of the native preparations corresponded to that previously observed[7]. In a material of 25 native aggregates the central filament measured on the average 1037 nm (range 341-2343 nm) with an average of 29 monomers attached at a mean interval of 36 nm. The average length of the monomers was 255 nm. Aggregates partially digested with chondroitinase displayed a similar general architechture while molecules digested with chondroitinase and trypsin or only trypsin appeared as filaments. The average length of these filaments was 442 and 864 nm respectively. HA isolated from nasal cartilage appeared as filaments with an average length of 732 nm. Assuming a M_w of about 2×10^6 for each monomer the average total M_w for PG aggregate from bovine nasal cartilage would be of the order of 6×10^7. The results of the observations and measurements on fragmented molecules and HA strongly corroborate the current model for the PG aggregate from hyaline cartilage. If the disaccharide unit of HA is assumed to have a length of 1 nm and a M_w of 416 the M_w of the HA of the aggregates of bovine nasal cartilage would be about 4.5×10^5, a figure in good agreement with the viscosity-average M_w of 5×10^5 recently estimated for HA from this tissue[10].

Summing up, electron microscopic studies on isolated proteoglycans have yielded information which confirms and extends available chemical and physical data. The technique should be applicable to investigations on proteoglycans from other tissues as well as subfractions of monomers and aggregates.

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