ASSOCIATION OF PROTEOGLYCAN WITH COLLAGEN FIBRILS IN THE BODY WALL OF THE SEA CUCUMBER (CUCUMARIA FRONDOSA)

John A. Trotter and Thomas J. Koob
Departments of Anatomy and Biology
University of New Mexico, Albuquerque, NM 87131

The body walls of sea cucumbers share with the collagenous tissues of other echinoderms the ability to undergo large alterations in stiffness and strength, which are thought to be due to changes in the ability of the interfibrillar matrix to inhibit the sliding of collagen fibrils past one another (Wilkie, 1984, Mar.Behav.Physiol. 11:1; Hidaka and Takahashi, 1983, J.exp.Biol. 103:1). Because the surfaces of the collagen fibrils and the matrix between them are thus apt to be important mediators of tissue mechanical properties we have begun to study the collagen fibrils and the macromolecules associated with them in the body wall of C.frondosa.

Intact collagen fibrils were isolated from the body wall of C.frondosa by a modification of the method of Matsumura (1974, Connect.Tiss.Res. 2:117). Isolated fibrils were exposed to cuprolinic blue under conditions in which it precipitates and stains sulfated polyanions (Scott 1988, Biochem.J. 252:313) prior to negative or positive staining with uranyl acetate (Trotter and Koob, 1989, Cell Tiss.Res. 258:527). The negatively stained fibrils have clearly delineated overlap and gap regions and show cuprolinic blue stained material associated with the gap (Fig.1, top). The repeat period (D-period) of the negatively stained fibrils measures 67.0±2.1nm, and the overlap/D ratio is 0.46±.03 (mean ± S.E.M.; N=20). After positive staining the isolated fibrils are seen to have 9 prominent subbands per D-period (Fig. 1,

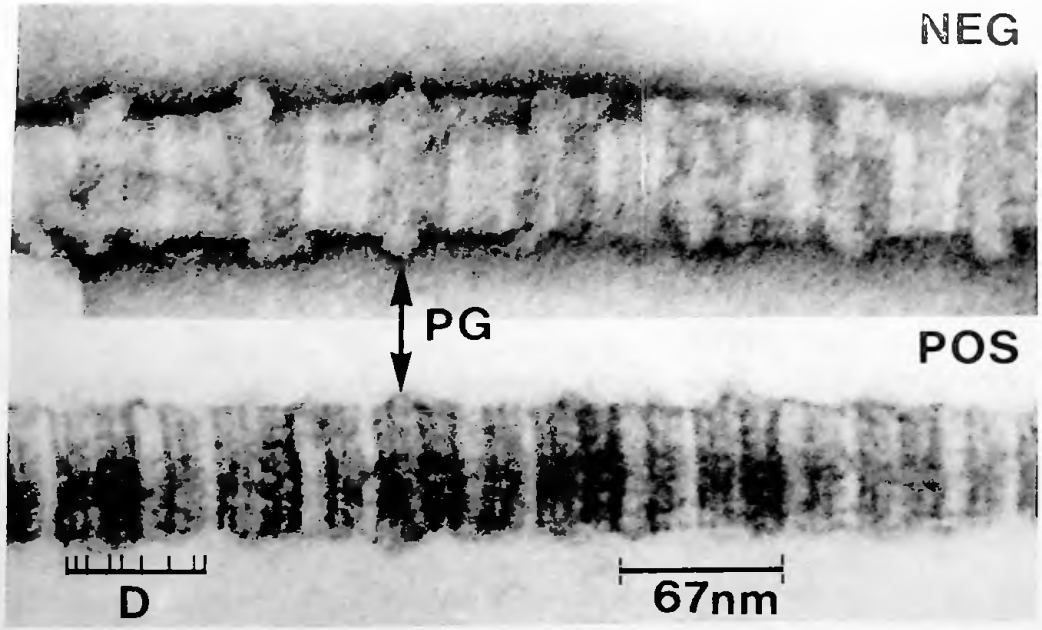

Figure 1. Isolated collagen fibrils stained with cuprolinic blue followed by negative (NEG) or positive (POS) staining with uranyl acetate. D:collagen repeat period; G:gap; O:overlap; PG:cuprolinic blue stained material.

bottom), consisting of a triplet, a doublet, a singlet, a singlet, and a doublet. The cuprolinic blue stained material is associated with the space between the triplet and the adjacent doublet, or with the triplet band nearest this space. Analysis of fibrils that have been both negatively and positively stained has permitted the two fibrils in Fig. 1 to be exactly aligned with one another, revealing that the gap region of the fibril consists of the triplet, the doublet, and the singlet, while the overlap region consists of the remaining singlet and doublet.

Previously Scott (op.cit.) has shown in sectioned material that the body wall of Stichopus japonicus contains polyanionic molecules, which he considers to be proteoglycans, that are precipitated and stained by cupromeronic blue (a stain closely related to cuprolinic blue) and are periodically associated with collagen fibrils in the gap region. The cuprolinic blue stained material associated with the gap region of C.frondosa fibrils may be proteoglycan, since the collagen associated proteoglycans of vertebrate tendon (Scott, op.cit.) and of sea urchin ligament (Trotter and Koob, op.cit.) are also localized by cuprolinic blue in the gap and are both associated with a specific subband. Furthermore, the banding pattern and cuprolinic blue localization in C.frondosa fibrils are identical to those of fibrils from E.tribuloides (Trotter and Koob, op.cit.).

Extracts of the body wall of C.frondosa were made in 4M GuHCl and were subsequently chromatographed on DE-52 ion exchange resin in 7M urea. SDS-PAGE analysis of the material that eluted between 0.2 and 1.0M NaCl revealed three bands that stained with alcian blue, one at the interface between the stacking and separating gel (about 500kDa), one with a relative molecular mass of about 340kDa, and a third that migrated at a position corresponding to about 71kDa. Incubation with chondroitinase ABC or keratinase did not affect the migration position of any of these bands, indicating that their glycosaminoglycan moieties are not simple chondroitin or keratin polymers. Treatment with Staph.aureus V8 protease did not affect the 500kDa or 71kDa band, suggesting that these are not proteoglycans but may be free glycosaminoglycans. The 340kDa band was degraded by V8 protease, producing an alcian blue stained band with an apparent molecular weight of approximately 280kDa. This suggests that 340kDa band is a proteoglycan. Its lack of sensitivity to chondroitinase and keratinase may be because of substitutions that block the activity of these enzymes, which has been demonstrated in a fucose sulfate substituted chondroitin-6-sulfate from the body wall of Ludwigotheria grisea (Vieira and Mourao, 1988, J.Biol.Chem. 263:18176), and S.japonicus (unpublished observations of H.Z.Fan and J.F.Kennedy, cited in Scott, op.cit.). It will be interesting to determine whether this 340kDa proteoglycan is the same molecule that stains with cuprolinic blue along the surfaces of the fibrils, and whether the mechanical alterations seen in the intact body wall can be mimicked by recombining its separated constituents under controlled conditions.

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