VISUALIZATION OF COLLAGEN IN FROG SARTORIUS MUSCLE BY X-RAY DIFFRACTION

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The author (1) has previously demonstrated the expansion of the major collagen equatorial spacing by urea from its normal value of 11.7 A to 12.9 A. The phenomenon was produced by soaking rat tail tendon under tension in a strong solution of urea, when the strongly hydrogen bonding urea replaced the water molecules in the space between the collagen spirals. On drying, the collagen spirals were propped apart by the vertically (along the fiber axis) oriented urea molecules, which also served as seed for oriented crystallization of urea along its c axis.

In an attempt to determine whether the lattice of other fibrous proteins could be modified by urea, frog sartorius muscle was suspended overnight in a 1M solution of urea in 0.65% NaCl, at 70 grams tension, dried for 24 hours and the X-ray diffraction pattern obtained, using Cu K-alpha radiation, with the fiber axis perpendicular. The diffractogram is shown in Figure 1.

The broad beta-myosin halos described by Astbury (2) are evident in the diffractogram with the inner halo at 9.8 A (range 8.16 to 11.45 A) and the outer halo at 4.42 (Astbury 4.65). In the absence of urea, the collagen equatorial spot at 11.7 A blends with the inner border of the inner halo (11.45 A) and is not distinguishable. After the treatment with urea, as described above, the collagen equatorial

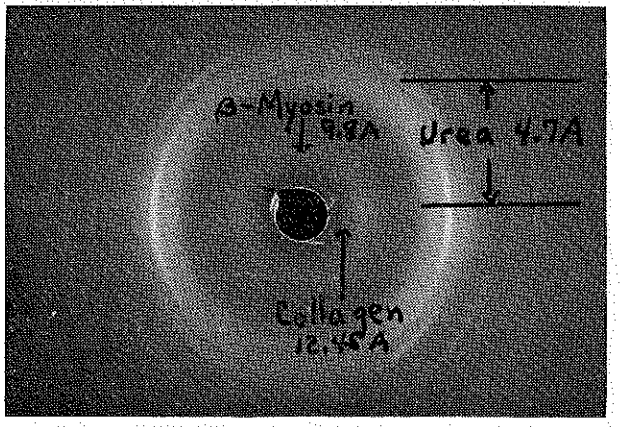

Figure 1. X-ray Diffractogram of Frog Sartorius Muscle as modified by Urea.

spot moves inward to 12.45 A (range 11.45 to 13.36 A) so as to be just inside the beta-myosin inner halo. The urea shows oriented crystallization on its c-axis, with rather broadly spread spots. It is not possible to determine whether the nucleus for crystallization was poorly oriented myosin fibers or the collagen sheath around each muscle fiber.

The phenomenon described suggests a possible use in determining the relative concentration of collagen in muscle by comparison of the optical density of the inner halo of muscle with that of the collagen spot. It is possible that other hydrogen bonding materials, such as glycerol, might give even better definition for the inwardly migrating collagen spot.

BIBLIOGRAPHY

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2. W. J. Astbury, Advances in Enzymology, *3*, 63 (1943).