Starch-gel Electrophoresis in the Characterization of Products of Collagen Breakdown

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The sharp resolution of the components of pepsin-digested collagen in starch-gel electrophoresis [1] prompted us to try this method to "finger-print" various collagen digests as an aid in structural analyses and comparisons. Acrylamide-gel electrophoresis has been used for the same purpose on cyanogen bromide (CNBr)-degraded collagen by Bornstein and Piez.[2] Fig. 1 shows three different patterns of partially degraded collagens, which differed either in the method of breakdown or in origin.

Salt-soluble collagen of lamprey (*Petromyzon*) skin [3] was dissolved in 0.5 % acetic acid to contain 0.34 % (w/v) of protein. A stock solution of pepsin containing 1 % (w/v) pepsin (1:60 000, twice crystallized, Sigma Chemical Company, Lot 54 B 1100) in 3 % acetic acid was shaken for 2 h at +4°C before it was diluted and an aliquot pipetted into collagen solution which had been denatured for 15 min at +40°C and then cooled to the digestion temperature (+25°C). Various enzyme-substrate ratios varying from 1:500 to 1:63 and digestion times from 1 to 48 h were employed. The ratio 1:250 and a period of 4 h were found suitable for degrading the *Petromyzon*-collagen. The starch-gel electrophoresis has been described earlier.[4,5] The gel-electrophoretic pattern of pepsin-digested collagen of lamprey differs markedly from that of rat-tail-tendon (RTT) collagen digested as described earlier.[1]

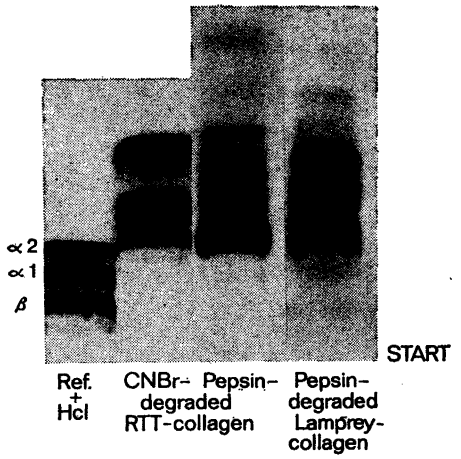

Fig. 1. Starch-gel electrophoretic patterns of CNBr-degraded rat-tail-tendon (RTT) collagen, pepsin-digested *Petromyzon* collagen, and pepsin-digested RTT collagen. The experimental details are described in the text.

The breakdown of RTT collagen by CNBr was performed at +25°C.[2,6] A final, typical pattern consisting of eight bands was obtained after 30 min treatment. Some low-molecular material had been formed because hydroxyproline was identified in the solution in the cathode vessel.

Preparative methods have been worked out for the isolation of the components of pepsin-digested RTT collagen (unpublished work), and they should be applicable to CNBr-degraded collagen also. Starch-gel electrophoresis is a useful, rapid means of surveying various degradation and fractionation products and also for comparing collagens from various species.

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