THE DIFFERENCES BETWEEN $\alpha 1$ AND $\alpha 3$ CHAINS OF ACID SOLUBLE CALFSKIN COLLAGEN

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Whereas the collagen molecule of acid-soluble rat-skin collagen is only composed of two different chains (2 $\alpha 1$ chains and 1 $\alpha 2$ chain) [1], there is evidence that acid soluble calfskin collagen consists of three different chains, $\alpha 1$, $\alpha 2$ and $\alpha 3$. Pikkarainen and Kulonen [2], Heidrich and Wynston [3] and François and Glimcher [4] have reported on three different chains in acid soluble calfskin collagen. Their findings agree in that the separation of $\alpha 1$ and $\alpha 3$ chains is very difficult, and that such a separation cannot be achieved by disc electrophoresis or by chromatography on CM cellulose, but only by starch gel or free-flow electrophoresis.

The existence of three peptide chains could, on the other hand, be clearly demonstrated by examining the molecular fragments obtained after treating acid soluble calfskin collagen with collagenase [5]. By this process the collagen molecules are attacked from their end regions and shortened. After denaturation it is possible to separate the two shortest fragments, with lengths of 1250 Å and 780 Å, into three components (the $\alpha 1$, $\alpha 2$ and $\alpha 3$ chain fragments) by means of disc electrophoresis or chromatography on CM-cellulose. The $\alpha 1$ and $\alpha 3$ chains of the 1250 Å and 780 Å fragments are very similar in both their amino acid composition and in their peptide maps. This suggests that the differences between the $\alpha 1$ and $\alpha 3$ chains are due to the presence of different peptide side chains and not to the amino-acid sequence of the main chains. It is known that by careful treatment with pronase, 5% of the total nitrogen is separated from the collagen molecule. Drake et al. [6] report that this material originates from the so-called telopeptides and from peptide side chains along the molecule.

In order to ascertain whether the differences between the $\alpha 1$ and $\alpha 3$ chains are to be found in the peptide side chains, acid-soluble collagen from calfskin which had been treated with pronase was submitted to collagenase digestion. The molecular fragments thus obtained were examined to determine the number of different peptide chains. The pronase treatment was carried out twice every 24 hours in 1 M calcium acetate at pH 7.8 at temperatures of 18 and 22°C. (Pronase, grade B, was obtained from Calbiochem. Corp., Los Angeles.) The pronase was subsequently removed by repeated precipitation of the collagen with 17.5% KCl. The collagenase treatment for the obtainment of molecular fragments and the characterization of

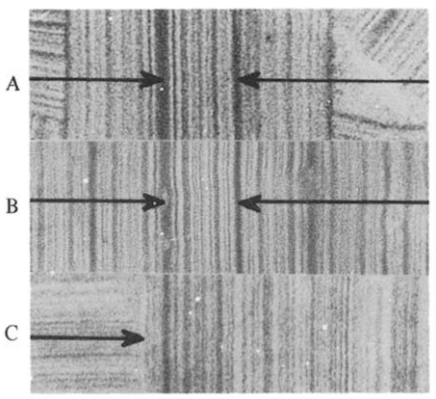

Fig. 1. Long spacing segments of the 780 Å molecular fragment of acid-soluble calfskin collagen: (A) from untreated collagen; (B) from pronase-treated collagen; (C) long spacing segment from intact collagen molecule (for comparison). The segments tend to end-to-end aggregation. Arrows indicate the ends of individual segments. The segments were precipitated by dialysis against 0.4% ATP solution of pH 2.9. They were stained with phosphotungstic acid and contrasted by treatment with uranyl acetate solution.

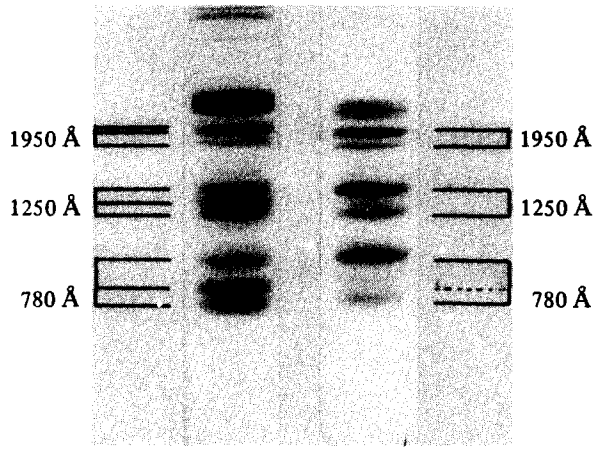

Fig. 2. Disc electropherograms of the molecular fragments obtained by collagenase treatment of acid-soluble calfskin collagen: (A) untreated collagen; (B) pronase-treated collagen. (Collagenase treatment: 0.5 M $CaCl_2$, pH 7, 10°C, 50 hr). Crude collagenase from Worthington Biochemical Corp., Freehold, N. J./USA, was used. For pronase treatment see text. Disc electrophoresis was carried out as described by Reisfeld [7] and Stark and Kühn [5].

these fragments by electron microscopic and disc electrophoretic investigations took place as reported by Stark and Kühn [5].

After collagenase treatment the collagen fragments were precipitated as segments with ATP. By means of electron microscopy no differences in these segments between the collagen treated with pronase and the untreated collagen could be detected. Both preparations give rise to the same molecular fragments. Fig. 1 shows, as an example, a comparison of the 780 Å fragments. Investigation by disc electrophoresis, on the other hand, showed characteristic differences. Whereas the 1250 and 780 Å fragments from untreated collagen separated into three distinct bands, only two bands could be recognized after treatment with pronase. The weaker the $\alpha 3$ band became, the more the $\alpha 1$ band increased in intensity. The still weakly visible band of $\alpha 3$ (780 Å) is due to an incomplete digestion of the peptide side chains by pronase.

These results suggest that the $\alpha 1$ and $\alpha 3$ chains from calfskin collagen differ in their peptide side chains, which can be split off by pronase, and not in the amino-acid sequence of the main chains. As the differences between the $\alpha 1$ and $\alpha 3$ chains become more pronounced with the decrease in length of the molecular fragments, it can be deduced that the peptide side chains that are responsible for the different electrophoretic mobility are located principally in the region of the 780 Å fragment.

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