ACTION OF HUMAN SKIN COLLAGENASE ON CARTILAGE COLLAGEN

D.E. WOOLLEY, R.W. GLANVILLE, K.A. LINDBERG*, A.J. BAILEY* and J.M. EVANSON
Department of Medicine, University Hospital of South Manchester, West Didsbury, Manchester 20, U.K.

and

**Agricultural Research Council, Meat Research Institute, Langford, Bristol, U.K.*

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1. Introduction

In contrast to most vertebrate collagens which have the molecular composition $[\alpha 1(I)]_2\,\alpha 2$, cartilage collagen has been shown to be largely composed of molecules which contain three similar α chains, designated α1(II), giving a molecular composition $[\alpha 1(II)]_3$ [1–3]. The former is known as Type I collagen and the latter Type II. Neutral collagenases are known to be active against Type I collagens and at temperatures below 27°C have been shown to cleave the collagen molecule into two fragments commonly called TC_A $(\frac{3}{4})$ and TC_B $(\frac{1}{4})$ [4–6]. However, its is not clear whether this attack is specific for Type I collagen. Although previous studies had shown that human neutral collagenase degraded pieces of intact cartilage [7, 8] it has recently been reported that soluble cartilage collagen is resistant to gingival and polymorphonuclear leucocyte collagenase [9]. We have therefore examined the effect of purified human skin collagenase on soluble cartilage collagen and report here the susceptibility of Type II collagen to attack by a neutral collagenase.

2. Materials and methods

2.1. *Preparation of human skin collagenase*

Skin explants were cultured in Dulbecco's modified Eagle's medium at 37°C for up to 10 days as previously described [6]. The neutral collagenase released into the culture medium was purified using a procedure based on gel filtration with Sephadex G-200 and G-100 superfine, and ion exchange chromatography employing Sephadex QAE, A-50. Non-specific protease activity was found to be totally absent in the final enzyme preparation as judged by its failure to degrade casein, [^{125}I]hemoglobin and [^{125}I]bovine serum albumin [10]; activity towards the synthetic peptide pZ–Pro–Leu–Gly–Pro–DArg, (Fluka Chemicals) was also absent [10, 11].

2.2. *Cartilage collagen*

Collagen was extracted and purified from the sternal cartilage of 3 week old lathyritic chicks as described by Miller [2]. The molecular composition $[\alpha 1(II)]_3$ was checked by amino acid analysis and polyacrylamide electrophoresis in sodium dodecyl sulphate (SDS).

2.3. *Viscometry*

Viscosity measurements were made using Ostwald viscometers of 1.0 ml capacity with water flow times at 25.0°C of 25–30 sec. The reaction mixture of 1.0 ml contained 0.73 mg collagen dissolved as tropocollagen molecules in a solution containing 0.17 M NaCl, 0.005 M $CaCl_2$ and 0.02 M Tris-HCl, pH 7.6. The collagen solutions were brought to 25.0°C and incubation was started by adding either trypsin (Sigma, Type I) bacterial collagenase (Form III, Advance Biofactures Corp., N.Y.) or purified skin collagenase, each dissolved in the same buffer.

2.4. *Disc gel electrophoresis*

Collagen cleavage products were examined in polyacrylamide gels containing SDS according to the method of Weber and Osborn [12].

3. Results and discussion

When lathyritic Type III cartilage collagen was incubated with skin collagenase a significant reduction in viscosity was observed as shown in fig. 1. The reaction was almost complete after 5 hr at 25.0°C and the final reading after 24 hr represented a 60% reduction in the original viscosity. Bacterial collagenase produced a reduction in viscosity amounting to approx. 95%. The collagen solution was resistant to non-specific protease activity and reaction mixtures containing trypsin consistently showed a reduction in viscosity of less than 5% after 24 hr of incubation.

In order to study the products of the reaction of skin collagenase with cartilage collagen at 25°C, the solution was examined by disc gel electrophoresis. A reaction mixture incubated for 24 hr and applied to polyacrylamide gels containing SDS produced two major components (fig. 2). These two protein bands represented molecular weights of approx. 70 000 and 25 000 daltons when calculated from SDS polyacrylamide gels calibrated with cyanogen bromide-cleaved collagen peptides of known molecular weight. The products therefore represent approx. $\frac{3}{4}$ and $\frac{1}{4}$ length fragments of the intact collagen molecule and are probably analogous to the TC_A and TC_B products

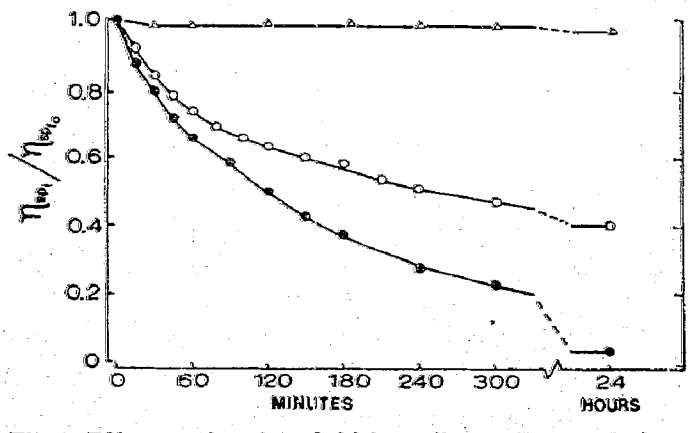

Fig. 1. Effect on viscosity of chick cartilage collagen solutions at 25°C of human skin collagenase, bacterial collagenase and trypsin. The reaction mixtures of 1.0 ml contained 0.73 mg collagen in 0.17 M NaCl, 0.005 M $CaCl_2$ and 0.02 M Tris-HCl, pH 7.60, with 70 μg trypsin (△—△—△), 0.16 mg purified skin collagenase (○—○—○) and 5 units bacterial collagenase (●—●—●).

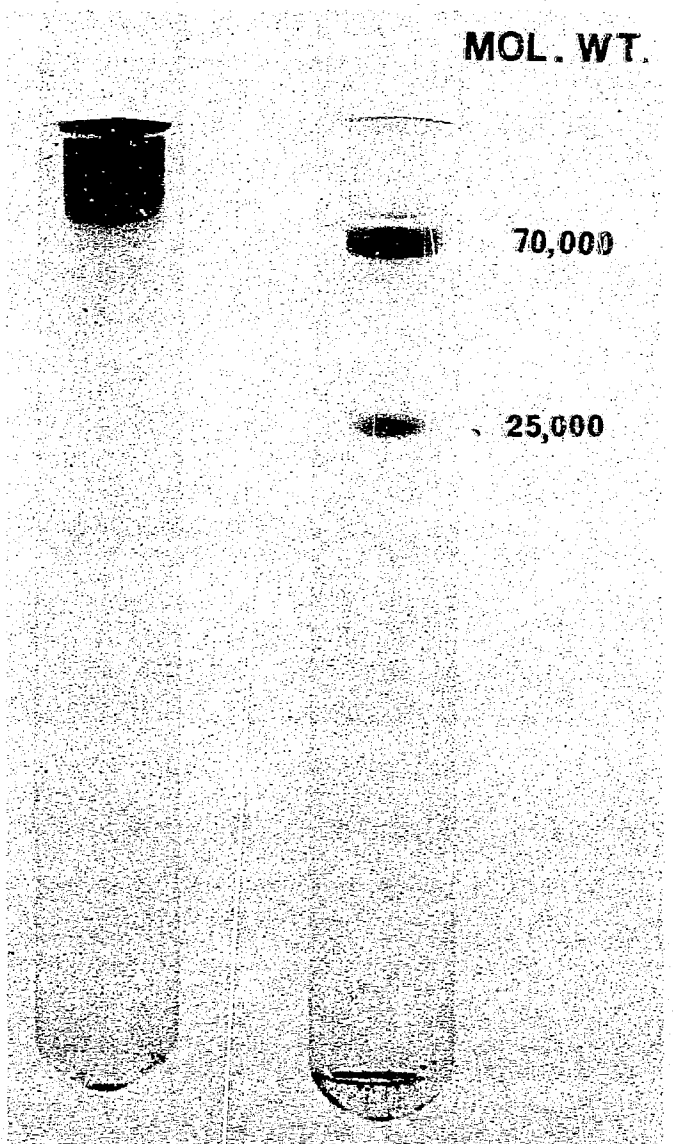

Fig. 2. Disc gel electrophoresis in 10% polyacrylamide containing SDS of cartilage collagen after exposure to skin collagenase for 24 hr at 25°C. The reaction products represent approx. $\frac{3}{4}$ and $\frac{1}{4}$ length fragments of the collagen molecule. Left: substrate alone; Right: substrate and enzyme.

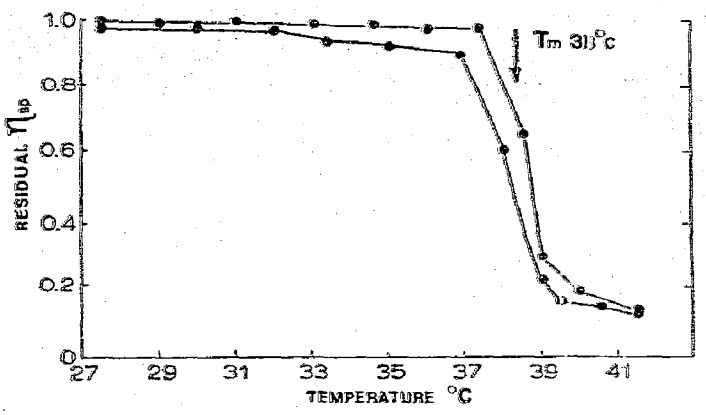

Fig. 3. Thermal denaturation of the reaction products obtained from cartilage collagen and skin collagenase inhibition at 25°C determined by viscometry. After inhibiting the enzyme the residual specific viscosities were obtained during stepwise increases in temperature. Results of two similar experiments are shown in which the Tm for the $\frac{3}{4}$:$\frac{1}{4}$ products was approx. 38°C.

commonly reported for collagenase-cleaved Type I collagens [4–6].

When the reaction between the skin collagenase and collagen was complete after 24 hr at 25°C EDTA was added to a final concn. of 0.01 M to inhibit the enzyme. The temperature of the reaction mixture was then elevated stepwise in increments of 1 or 2C° and each temperature maintained for 15 min after which time the viscosity was read. Fig. 3 shows that the mid-point melting temperature (Tm) of the reaction products determined in this way was approx. 38°C. This Tm for the $\frac{3}{4}$:$\frac{1}{4}$ products of the chick cartilage collagen is very similar to that reported for intact collagen molecules of chick and bovine articular cartilage [1, 2] but is about 3°C higher than the Tm reported for TC_A and TC_B fragments of Type I guinea pig skin collagen [4]. Whether or not this disparity is accounted for by collagen type or phylogenetic differences has still to be examined.

Although it seems clear from the electrophoretic studies that skin collagenase will take to completion the cleavage of the Type II collagen monomers into large and small fragments, we have some preliminary evidence that the rate of cleavage differs with the Type I and II collagens, the former being more rapidly attacked than the latter.

In any event, unlike the reported resistance of pepsin-solubilized cartilage collagen to gingival and polymorphonuclear leucocyte collagenases [9], the absence of an α2 chain and the primary structure of the α1(II) chain do not apparently prevent purified human skin enzyme from cleaving the molecule in a manner similar to that for Type I collagen.

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