Inhibition by Pepstatin of Human Cartilage Degradation

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Immunoenzymic experiments have demonstrated a major role of cathepsin D in the autolysis of chicken and rabbit cartilage (Dingle et al., 1971), and human cathepsin D has been shown capable of degrading human cartilage proteoglycans (R. I. G. Morrison, A. J. Barrett & J. T. Dingle, unpublished work). The results of experiments made with inhibitory antisera to human cathepsin D have demonstrated substantial inhibition of autolysis, but, because of the possibility of non-specific inhibition of proteinases, other than cathepsin D, by normal serum components, it has not been possible to fully quantitate the role of cathepsin D in this system. Pepstatin has now been shown to be a very potent inhibitor of human and rabbit cathepsin D (Barrett & Dingle, 1972). It therefore seemed likely that pepstatin would inhibit any part of the autolysis of human cartilage that was due to cathepsin D, allowing the participation of any other enzymes in this process to be clearly demonstrated.

Materials and methods

Human adult articular cartilage, healthy in appearance, was obtained at autopsy, and stored at −20°C until required. Rabbit ear cartilage was taken from animals 6 weeks of age, immediately after death.

The release of degraded proteoglycan during autolysis was measured by the turbidimetric method of Dingle et al. (1971), chondroitin sulphate being used as standard.

Experimental

The pH optimum for the autolysis of adult human cartilage, like that of human embryonic cartilage R. I. G. Morrison, A. J. Barrett, J. T. Dingle & D. C. Prior, unpublished work) and chicken and rabbit cartilage (Dingle et al., 1971), is pH 5.0 (Fig. 2). Incubation at this pH and 37°C for 24h resulted in the release of approximately 50% of the cartilage proteoglycan. The addition of pepstatin to a concentration of 0.1 μg/ml completely inhibited this release (Fig. 1), and 90% inhibition was obtained with as little as 0.01 μg/ml. A concentration of 0.1 μg/ml was found completely to inhibit the autolysis of rabbit ear cartilage, under similar conditions.

With some human cartilage samples, pepstatin completely inhibited autolysis over the pH range 3–8 (as is shown in Fig. 2) in the absence of exogenous activators of thiol-dependent enzymes. With other samples, however, there was a trace of pepstatin-resistant activity in the region of pH 6, and this

![Fig. 1. Effect of pepstatin on the autolytic release of proteoglycan from cartilage](image)

Chopped human articular cartilage (40 mg wet wt.) from the femoral head of a 40-year-old male was twice frozen and thawed and then incubated for 24h at 37°C in 500 μl of medium containing 40 μl of 0.2 M-sodium acetate buffer, pH 5.0, and 100 μl of pepstatin to a final concentration of 0.004–2 μg/ml. (Concentrations of pepstatin above 0.2 μg/ml were completely inhibitory and are not shown in the figure.) The results are the means of duplicate determinations on each of three replicates at each concentration. Maximum variation of the determined values occurred in the absence of pepstatin; the standard deviation on a release of 425 μg was 16 (six samples). The release of proteoglycan was measured turbidimetrically and expressed as μg of chondroitin sulphate.
became much more pronounced in the presence of dithiothreitol (1 mM) and EDTA (1 mM) (Fig. 2). At pH 6, the activatable activity amounted to about 20% of that attributable to cathepsin D at its optimum of pH 5. We conclude that in the autolysis of human articular cartilage proteoglycan, as in that of other species, cathepsin D is the most active enzyme. However, the relative importance of cathepsin D and the thiol-dependent enzyme(s) in the turnover of proteoglycans in the living tissue remains to be established.

The pepstatin-resistant enzymic activity at pH 6.0 was 59% inhibited by iodoacetate (1 mM) and by 7-amino-1-chloro-3-tosylamidoheptan-2-one ('tosyl-L-lysine chloromethyl ketone') (1 mM), whereas normal sheep serum (50%, v/v) produced 30% inhibition. In each of these experiments the inhibitor (sufficient to give the final concentration stated) was pre-incubated with the tissue in 450 µl of buffer containing 0.2 µg of pepstatin at 0°C for 90 min before the addition of 50 µl of dithiothreitol (10 mM) with EDTA (10 mM). The results are consistent with part of the pepstatin-resistant activity being due to cathepsin B1 (in the terminology of Barrett & Dingle, 1971), since this enzyme is known to be inhibited by iodoacetate and 7-amino-1-chloro-3-tosylamidoheptan-2-one (Keilová, 1971), and by human α2-macroglobulin (P. M. Starkey & A. J. Barrett, unpublished work). It is significant that R. I. G. Morrison, A. J. Barrett & J. T. Dingle (unpublished work) have shown that pure cathepsin B1 is capable of degrading human cartilage proteoglycan.

If cathepsin D plays an important part in the normal turnover of proteoglycans in human articular cartilage and in their accelerated breakdown in arthritis, perhaps synergistically with other proteinases, it seems possible that pepstatin, or some related inhibitor, may be applicable to the local control of this process.