Biochimica et Biophysica Acta, 658 (1981) 138—147

BBA 69227

NEUTRAL PROTEINASES FROM ARTICULAR CHONDROCYTES IN CULTURE

2. METAL-DEPENDENT LATENT NEUTRAL PROTEOGLYCANASE, AND INHIBITORY ACTIVITY

ASHER I. SAPOLSKY [a,*], CHARLES J. MALEMUD [b,**], DAVID P. NORBY [b], ROLAND W. MOSKOWITZ [b], KUNIO MATSUTA [a] and DAVID S. HOWELL [a]

[a] *Departments of Medicine and Biochemistry, University of Miami School of Medicine, Miami, FL and* [b] *The Cartilage Research Laboratory, Departments of Medicine and Orthopedics, Case Western Reserve University, Cleveland, OH (U.S.A.)*

(Received March 20th, 1980)
(Revised manuscript received November 24th, 1980)

Key words: Proteoglycanase; Proteinase; Metal ion dependence; (Chondrocyte)

Summary

Monolayer and spinner cultured rabbit articular chondrocytes released into the medium latent metal-dependent enzyme with activity against bovine proteoglycan. Pretreatment of medium with *p*-aminophenylmercuric acetate or trypsin followed by soybean trypsin inhibitor significantly increased enzyme activity. The monolayer-cultured chondrocytes released more of this activity than spinner cultures.

The neutral proteoglycanase activity increased with medium concentration and incubation time. Like the human cartilage proteoglycanase, its pH optimum on proteoglycan subunit was 7.25. Gel filtration on BioGel P-30 indicated that the proteoglycanase occurred in two molecular weight forms: 20 000—30 000 and 13 000. The latent enzyme was about 30 000—40 000. The metal-chelators, *o*-phenanthroline (5 mM) and EDTA (10 mM) inhibited the activated proteoglycanase almost completely, but trypsin and chymotrypsin inhibitors had little effect.

The cultured chondrocytes also released into the media a heat-labile inhibitor against the proteoglycanase. The inhibitory activity was present in the non-activated media and eluted on Sephadex G-100 chiefly at a position corresponding to molecular weights of 10 000—13 000.

* Present address: Miriam Hospital Research Center, Providence, RI, U.S.A.
** To whom reprint requests should be addressed.

Introduction

The primary events which trigger the destruction of cartilage in joint disease remain unknown. However, it is generally recognized that the destruction of the chief components of the cartilage matrix, collagen and proteoglycan, must be carried out by proteolytic enzymes [1,2]. Until recently most studies focused on the proteinases originating from synovial fibroblasts [3], macrophages [4] and polymorphonuclear leukocytes [5] which were assumed to invade the neighboring cartilage under pathological situations. It seemed, however, that although these enzymes probably played a primary role in inflammatory type arthritides, they probably serve only a secondary one in osteoarthritis. In osteoarthritis, loss of proteoglycan occurs long before appearance of clinical symptoms [6] indicating that the initial attack on the matrix proteoglycan may come from within the cartilage matrix. The acid protease cathepsin D, known to be present in cartilage, was assumed to play this role, but the discovery of its inhibitor pepstatin proved that it could not act at the neutral pH of the cartilage matrix and that its role in proteoglycan degradation must be limited to within or in the immediate vicinity of the chondrocytes [7]. Sapolsky et al. [8] identified a metal-dependent neutral protease in human articular cartilage which degraded proteoglycan optimally at the neutral pH of the cartilage matrix. The supposition that this proteoglycanase activity most likely comes from the chondrocytes within the cartilage was supported by our subsequent finding that rabbit chondrocytes in monolayer culture secreted into the medium similar metal-dependent neutral proteoglycanase activity [9].

Deshmukh-Phadke et al. [10] have shown that a lipopolysaccharide-activated macrophage factor stimulated release of a collagenase and neutral nonspecific protease by chondrocytes. Activity was inhibited by EDTA but not by soybean trypsin inhibitor. This report and the recent finding that a factor derived from synovial tissue induced the chondrocytes in porcine cartilage explants to extensively degrade their own cartilage matrix [11,12] emphasizes the significant role chondrocyte enzymes may have in initiating cartilage degradation within the cartilage matrix.

In the aforementioned investigations enzyme induction by chondrocytes resulted from treatment by factors from outside the cartilage. In the present studies no such outside stimulation was needed. However, only a small amount of intrinsic proteoglycanase activity was measured. This finding correlates with the small amount of enzyme extracted from a very large amount of the human articular cartilage [8]. The present investigations also revealed that the rabbit chondrocytes, in both monolayer and spinner cultures, did indeed produce, in addition to the previously reported active proteoglycanase [9], a significantly greater amount of latent metal-dependent neutral proteoglycanase and also inhibitor(s) of this activity.

Materials and Methods

Materials. All culture media, balanced salt solutions, antibiotics, antimycotics and fungizone were obtained from Grand Island Biological Co., Grand

Island, NY, U.S.A. Fetal bovine serum (not heat-inactivated) was purchased from Associated Biomedic Systems, Buffalo, NY, U.S.A. Trypsin and soybean trypsin inhibitor was obtained from Worthington Biochemicals, Freehold, NJ, U.S.A. Lactalbumin hydrolysate was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. BioGel P-30 was purchased from BioRad Laboratories, Richmond, CA, U.S.A. and Sephadex G-100 was obtained from Pharmacia, Inc., Piscataway, NJ, U.S.A. All chemicals were of reagent or the best available grade.

Chondrocyte culture. Three separate monolayer and two spinner chondrocyte culture media were prepared (monolayer I, II, III, spinner I and II) as described in detail elsewhere [9,13,14], The chondrocytes were obtained from the pooled articular cartilage of young immature New Zealand White rabbits as previously described [13]. Before assay, the medium was lyophilized, the lyophilized powder was dissolved (generally 100 mg/ml) in 5 mM Tris-HCl buffer, pH 7.25/0.02% NaN_3, and dialyzed in dialysis bags with 2000 molecular weight cut-off (Spectrum Medical Industries, Inc., Los Angeles, CA) against 100 vol. 5 mM Tris-HCl buffer, pH 7.25/0.5 mM $CoCl_2$/0.02% NaN_3 at 4°C. In these experiments inclusion of $CoCl_2$ during dialysis retained the proteoglycanase activity more consistently than when medium was dialyzed against buffer containing $CaCl_2$.

Proteoglycanase assay. Proteoglycan subunit used as the substrate for the proteoglycanase assay was prepared from bovine nasal septum cartilage by the single-step dissociation method of Hascall and Sajdera [15] and lyophilized. A small amount of substrate was dissolved (10 mg/ml) in 0.05 M Tris-HCl buffer, pH 7.25/0.02% NaN_3/200 μg penicillin/250 μg streptomycin/ml and kept at 4°C. The proteoglycan subunit substrate was preincubated 10 min at 37°C before addition of the sample, since preincubation resulted in better constant viscosity of proteoglycan subunit blanks. Dialysed media samples or buffer for blanks (50 μl) and proteoglycan subunit (150 μl) were incubated directly in microviscometers as previously detailed [16]. Inhibitors, made to pH 7.25, were preincubated with the samples or blanks for 15 min at room temperature before adding the proteoglycan subunit. The percent loss of viscosity was measured as previously described [8] and obeys second-order kinetics. This was converted into the corresponding second-order rate constant and expressed as units. The second-order rate constants were obtained by use of the relation $t^{-1}(N_o - N_t)/(N_o - N_\infty)$, where N_o, N_t and N_∞ are the flow times of the solutions at time o, t and complete digestion of the proteoglycan subunit. For example, when the change from N_o to N_∞ is 50% complete the rate constant $K = 1$, and when 75% complete, $K = 3$. The flow time in a particular viscometer for proteoglycan subunit completely degraded by the proteoglycanse was determined experimentally ($K = 1 = 100$ units).

Activation of the latent enzymes. Medium was preincubated with trypsin followed by soybean trypsin inhibitor to terminate the action of trypsin or *p*-aminophenylmercuric acetate. For activation by trypsin the medium was incubated with 100 μg/ml trypsin for 10 min at 37°C, cooled to 4°C, and then 500 μg/ml soybean trypsin inhibitor was added. The mixture was kept at room temperature for 15 min before assaying. *p*-Aminophenylmercuric acetate activation was carried out by incubating medium with *p*-aminophenylmercuric

acetate (generally 0.5 mM) for 15 min at room temperature. The optimal amount of *p*-aminophenylmercuric acetate (0.25—1.0 mM) was determined for each medium batch. A 20-fold concentrated stock solution of *p*-aminophenylmercuric acetate was prepared by dissolving it at alkaline pH and reducing the pH gradually with 0.1 N HCl. Blanks were run with proteoglycan subunit plus *p*-aminophenylmercuric acetate or trypsin-soybean trypsin inhibitor mixture.

pH Optimum curve. The curve of proteoglycanase activity vs. pH was made with proteoglycan subunit dissolved in the following buffers: 0.05 M Tris-HCl buffer, pH 7.25—8.5; 0.05 M phosphate buffer, pH 6.5—7.25 and 0.05 M acetate buffer, pH 4.5—6.5 inclusive.

Gel filtration chromatography. Gel filtration was carried out on BioGel P-30, P-60 and Sephadex G-100 at 40°C. 3 ml monolayer medium (combined 1.5 ml each of monolayer I and monolayer III, 300 mg/ml), activated or non-activated, was placed on the column (100 × 0.6 cm) and eluted with 0.005 M Tris-HCl buffer, pH 7/0.1 M NaCl/0.005 M $CaCl_2$/0.02% NaN_3. Selected pooled eluate fractions were dialyzed against distilled H_2O + 0.02% NaN_3 at 4°C, lyophilized and dissolved in 1 ml 0.005 M Tris-HCl buffer, pH 7.25/ 0.005 M $CoCl_2$/0.02% NaN_3 prior to assaying for proteoglycanase activity. Molecular weight markers used were ovalbumin (42 000), carbonic anhydrase (29 000), chymotrypsinogen (25 000), soybean trypsin inhibitor (22 000) and cytochrome *c* (13 000). Void volume was determined with Blue Dextran.

Results

Proteoglycanase activity

A small amount of active neutral protease with proteoglycan-degrading activity at pH 7.25 was already present in all the chondrocyte culture media

TABLE I

ACTIVATION OF CHONDROCYTE LATENT NEUTRAL PROTEOGLYCANASE ACTIVITY

Units of proteoglycanase activity in the activated medium includes the nonactivated units. AMPA, *p*-aminophenylmercuric acetate; TRI, trypsin-soybean trypsin inhibitor.

Culture medium	Incubation (h)	Proteoglycan viscosity loss units					Percent inhibition by 5 mM *o*-phenanthroline of the proteoglycanase in the culture medium	
		Non-activated intrinsic activity	After activation by					
			APMA			TRI		
			0.25 mM	0.5 mM	1.0 mM		0.5 mM APMA	TRI
Monolayer I	1	14	—	128	—	59	95	93
75 mg/ml	20	47	—	550	—	321	95	94
300 mg/ml	1	25	—	—	62	—	—	—
	20	29	—	—	300	—	—	—
Spinner I	1	9	22	22	14	—	—	—
100 mg/ml	20	15	54	39	14	—	76	—

before the latent enzyme was activated (Table I). Less was present in the spinner than in the monolayer media. As previously reported [9], this nonlatent protease activity was only partially inhibited by 5 mM of the metal-chelator *o*-phenanthroline (50—78%, inhibition in 1 h incubation).

The chondrocyte culture media contained 3—10-fold more latent than intrinsic proteoglycanase activity as revealed by the activation with *p*-aminophenylmercuric acetate or trypsin (Table I). Since, both commercial trypsin and soybean trypsin inhibitor from several sources were contaminated with traces of proteoglycanase activity and produced higher blanks than the *p*-aminophenylmercuric acetate blank, most of the activations were carried out with *p*-aminophenylmercuric acetate. As in the case of the nonlatent intrinsic enzyme activity, the spinner media had considerably less latent proteoglycanase activity than the monolayer media.

In contrast to the partial inhibition of the nonlatent proteoglycanase, the latent proteoglycanase in the monolayer media, whether activated by *p*-aminophenylmercuric acetate or trypsin, was inhibited by 5 mM *o*-phenanthroline 93—95%; thus, it was almost entirely metal-dependent. The activated enzyme in the spinner media was inhibited only 76%. The activated proteoglycanase in the monolayer media was inhibited 92% by 10 mM EDTA, 11% by 10 mM phenylmethylsulfonyl fluoride, 10% by 10 mM tosyl-L-phenylalanine chloromethylketone and not at all by 10 mM iodoacetamide or soybean trypsin inhibitor.

The amount of *p*-aminophenylmercuric acetate used for the activation was critical (Fig. 1). In most of the monolayer media 0.5 mM *p*-aminophenylmercuric acetate was satisfactory while 0.25 mM caused little activation. On the other hand in the spinner media with much less latent enzyme; 0.25 mM was more effective than 0.5 mM (data not shown). Increasing the time of pre-

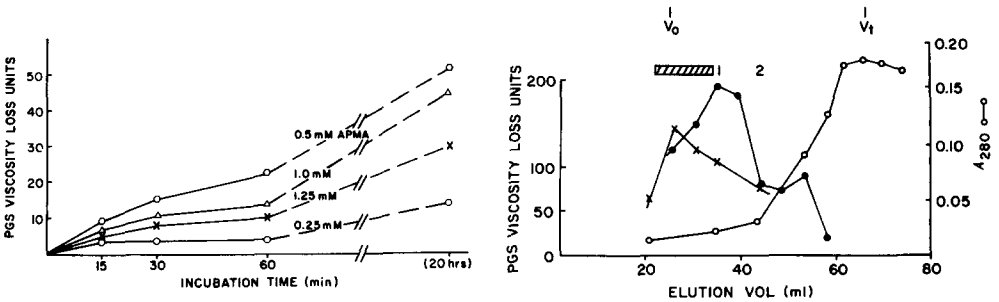

Fig. 1. Activation of monolayer III by *p*-aminophenylmercuric acetate. Monolayer III (100 mg/ml) was preincubated for 15 min at room temperature with sufficient concentrated *p*-aminophenylmercuric acetate stock solution to produce the required mM *p*-aminophenylmercuric acetate in the monolayer solution. PGS, proteoglycan.

Fig. 2. Gel filtration chromatography on BioGel P-30 of activated and nonactivated monolayer medium. Proteoglycanase activity was assayed by microviscometry after 48 h incubation with proteoglycan subunit (PGS) at 37°C of nonactivated monolayer, (X———X), or monolayer activated by *p*-aminophenylmercuric acetate, (•———•). Protein measured by absorbance at 280 nm. The horizontal bar shows the elution position of maximum collagenase activity obtained by chromatography of *p*-aminophenylmercuric acetate-activated monolayer I by assay of pooled fractions for activity against cartilage Type II collagen [17]. Standard protein markers; (1) carbonic anhydrase (29 000) and (2) soybean trypsin inhibitor (22 000). V_o, void volume. V_t, included volume.

incubation with the *p*-aminophenylmercuric acetate did not alter the extent of enzyme activation. Optimal preincubation time proved to be 15—30 min.

Chromatography of the activated monolayer medium on BioGel P-30 indicated that the activated proteoglycanase eluted in a peak between the 20 000 and 29 000 molecular weight markers and also a smaller peak at the 13 000 molecular weight marker (Fig. 2). Collagenase activity, which degraded human collagen type II at 24°C [17], eluted just in front of the main proteoglycanase peak. Most of the protein present in the medium eluted beyond the major proteoglycanase peak, and consisted largely of small molecular weight protein. As a result, the proteoglycanase activity was enriched 28-fold by the gel filtration.

Gel filtration of the nonactivated medium on BioGel P-30 produced a smaller peak in the same general elution position as the major activated proteoglycanase peak (Fig. 2). This smaller peak presumably was active enzyme already present in the 'nonactivated' medium. A somewhat larger peak of proteoglycanase activity between the void volume and the 29 000 molecular weight marker was also discerned, which may be another enzyme activity.

Gel filtration on Sephadex G-100 of the nonactivated monolayer medium produced three very small active proteoglycanase peaks, one centering at about the 35 000, one at 25 000 and one at the 13 000 molecular weight elution point (Fig. 3). When aliquots of these fractions were treated with 0.5 mM *p*-aminophenylmercuric acetate, the fractions eluting between the 40 000 and 25 000 molecular weight elution points became activated about 4-fold with the peak centering between the 25 000 and 29 000 molecular weight markers (Fig. 3).

The pH optimum curve of the proteoglycanase activity in a combined sample of monolayer I, II, III and spinner II activated by *p*-aminophenylmercuric acetate, showed optimum activity at pH 7.25, the same pH optimum as that of the human cartilage proteoglycanase, with similar low activity at alkaline pH [8].

TABLE II

PERCENT INHIBITION OF NEUTRAL PROTEOGLYCANASE IN THE ACTIVATED CULTURE MEDIA BY NONACTIVATED MEDIA

Monolayer media were activated by 0.5 mM *p*-aminophenylmercuric acetate. % Inhibition = 100 — (Proteoglycan subunit viscosity loss units with inhibitor × 100)/(Proteoglycan subunit viscosity loss units without inhibitor). Incubation time of activated monolayer I with nonactivated medium as indicated.

Sample tested (mg/ml)	Percent inhibition of activated monolayer I (100 mg/ml)		
	Incubation time		
	30 min	1h	3 h
Monolayer I 100	58	37	—
Monolayer I 200	97	89	—
Monolayer II 200	70	65	63
Monolayer II 200 heated *	12	10	0
Spinner II 200	70	25	14

* Heated at 100°C for 5 min.

Inhibitory activity

Incubation of the activated medium with nonactivated medium resulted in inhibition of the activated medium. This inhibition was directly dependent on the amount of nonactivated medium utilized. The inhibitory activity was present in all the nonactivated chondrocyte media in varying amounts. It was eliminated by the *p*-aminophenylmercuric acetate activation and by heating the medium for 5 min at 100°C (Table II).

When fractions eluting at or just beyond the 13 000 molecular weight point from Sephadex G-100 chromatography were incubated with activated monolayer medium, the activated proteoglycanase activity was inhibited 70% (Fig. 4). When this fraction was heated for 5 min at 100°C most of this inhibition was destroyed. A second, smaller inhibitory fraction eluted at about the 40 000 molecular weight point which inhibited the proteoglycanase in the medium by 45% (Fig. 4). Similarly, 2 inhibitory peaks were produced by gel filtration of the nonactivated monolayer medium on BioGel P-60 (data not shown). The larger peak eluted just beyond the 13 000 molecular weight point and inhibited the activated medium proteoglycanase by 80%, the smaller one eluted around the 40 000 molecular weight point and inhibited the proteoglycanase by 50%.

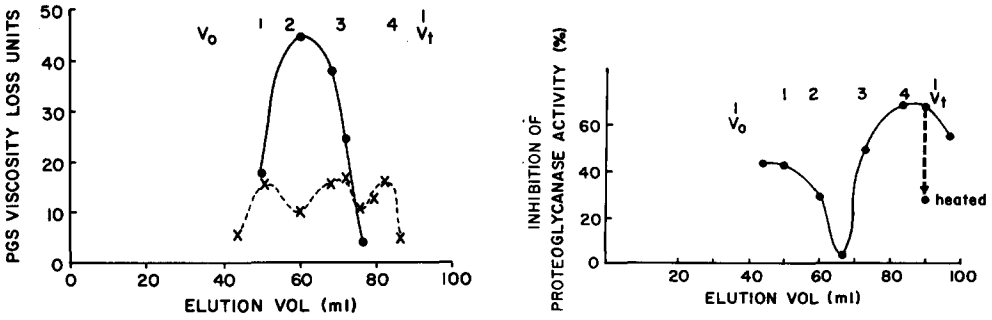

Fig. 3. Gel filtration chromatography of activated and nonactivated monolayer medium on Sephadex G-100. Proteoglycanase activity of nonactivated eluants (X- - - - -X) and after their activation by *p*-aminophenylmercuric acetate (•———•). All assays after 1 h incubation with proteoglycan subunit (PGS). The Sephadex G-100 chromatographic column (100 × 0.6 cm) was calibrated with standards and elution positions indicated as follows: (1) ovalbumin (42 000 daltons); (2) carbonic anhydrase (29 000 daltons); (3) chymotrypsinogen (25 000 daltons); (4) cytochrome *c* (13 000 daltons). Voild volume, V_o was determined with Blue Dextran 2000. V_t, included volume.

Fig. 4. Gel filtration chromatography of nonactivated monolayer medium on Sephadex G-100: Inhibition of proteoglycanase activity in *p*-aminophenylmercuric acetate-activated monolayer medium I by Sephadex G-100 eluant fractions of nonactivated monolayer medium. Individual chromatographic fractions (2 ml/fraction) were tested for their ability to inhibit *p*-aminophenylmercuric acetate-activated proteoglycanase activity. First 50 µl activated medium were mixed with 50 µl buffer and preincubated for 10—15 min at room temperature. After a 1 h incubation with proteoglycan subunit, the proteoglycanase activity was assayed by microviscometry. Then, 50 µl activated monolayer medium were preincubated with 50 µl of the gel filtration fractions, mixed with proteoglycan subunit, for 1 h, and the proteoglycanase activity of this mixture assayed. The percent inhibition of activity was calculated as given in Table II. Heated fractions were treated for 5 min at 100° C. Marker proteins are numbered as given in Fig. 3.

Discussion

Articular chondrocytes cultured under monolayer and spinner conditions released into the culture medium significant amounts of latent proteoglycan-degrading enzyme which, when activated by *p*-aminophenylmercuric acetate or trypsin, digested proteoglycan subunit at 37°C at neutral pH. Also present in the medium were small amounts of this or similarly acting enzymes in already active form. It was uncertain whether this latter enzyme was activated before or after its release by the chondrocytes. These nonlatent activities were inhibited by *o*-phenanthroline only about 50—70%, perhaps because minute amounts of nonlatent trypsin and chymotrypsin-type enzymes were also present in the medium [9]. The latent enzyme, however, was practically all metal-dependent; since it was inhibited almost completely by *o*-phenanthroline (Table I) and EDTA, and very little or not at all by standard serine protease and thiol-enzyme inhibitors.

Prior to finding that the human cartilage proteoglycanase was metal-dependent, mammalian metalloproteinases besides collagenases, were thought rare. Recently, however, neutral metalloproteinases have also been found in cultured rabbit synovial fibroblasts and granulocytes (25 000 daltons) [18], mouse bone explants [19], rabbit bone explants [20], cultured rabbit bone marrow macrophages [4], rabbit chondrocyte culture stimulated by a macrophage factor [10], bovine involuting uterus (Sellers, A., personal communication) and in the presently reported monolayer and spinner rabbit chondrocyte cultures. All these enzymes occurred chiefly in the latent form and in most cases concurrently with latent collagenase. It appears that these enzymes may occur generally where both collagen and other proteins undergo catabolic turnover.

The proteoglycanase activity released by the cultured chondrocytes resembled the metal-dependent proteoglycanase isolated from human articular cartilage in several respects [8]. Both were capable of extensive degradation of proteoglycan up to a similar limit of 80% loss of the original substrate viscosity. Both had a pH optimum of 7.25 on proteoglycan subunit. The enzyme activity could be resolved into two molecular weight forms in each preparation as was previously reported for the cartilage enzyme [21]. The chondrocyte proteoglycanase occurred as a major component (20 000—30 000 daltons) and a minor one (approx. 13 000 daltons). It remains to be determined whether these represent monomer and dimer forms as appears to be the case in the cartilage enzyme and whether the disaggregation and reaggregation phenomena observed with the cartilage enzyme also occurs here [22].

Besides the proteoglycanase, the chondrocytes released concurrently a heat-labile inhibitor of this enzyme into their culture media. The inhibitor was present in both nonactivated and activated spinner and monolayer culture media in varying amounts, but less in the spinner media (Table II). The results suggest that the amounts of proteoglycanase and inhibitor released depend on the culture conditions and that the more proteoglycanase is released the more inhibitor is also released (Tables I and II). Activation with *p*-aminophenylmercuric acetate destroyed the entire inhibitory activity in the media, indicating the enzyme latency may be due to formation of an enzyme-inhibi-

tor complex. If this is the case, then the inhibition by the nonactivated media of the activated enzyme must be due to the presence of excess inhibitor. This would suggest that the small amount of the nonlatent proteoglycanase in the media may be a different or transformed enzyme; or, perhaps, the complex itself is inhibitory as well.

Recently, gel filtration chromatography of extracts of bovine nasal cartilage showed distinct inhibitor activities against collagenase, thiol and serine proteinases (22 000, 13 000 and 7000 daltons, respectively) [23]. Human intervertebral disc and femoral head articular cartilage have been reported to contain small molecular weight substances which inhibit degradation of proteoglycan by trypsin, chymotrypsin, leukocyte elastase and cathepsin G [24]. The low molecular weight inhibitor reported by Kuettner et al. [25] from nasal, costal and articular cartilage acts against collagenase and serine proteases. These inhibitors may function to keep these proteases which abound in the synovial area from causing vascularization of and damage to the avascular cartilage from outside, and also to control the trace amounts of such enzymes which the chondrocytes may release inside the cartilage. In contrast, the presently reported inhibitor can act against the neutral metal-dependent proteoglycanase secreted by the same chondrocytes. Thus, inhibitor secreted by chondrocytes may function to control proteoglycan degradation orginating in the cartilage itself.

As suggested above, the enzyme latency may be due to formation of an inhibitor-enzyme complex, since both enzyme and inhibitor were released into the same medium and activation destroyed the inhibitory activity of the medium. However, this remains conjecture until the activation process is further clarified. Activation by *p*-aminophenylmercuric acetate may involve break up of thiol bonds in the inhibitor or enzyme or both. Gel filtration of the nonactivated medium isolated both latent enzyme, active enzyme and inhibitor fractions. The isolated inhibitor may be, in part, excess inhibitor in the nonactivated medium and in part, inhibitor liberated from partial break up of the complex by the gel filtration. The active enzyme may include the small amount of active enzyme in the medium and also enzyme liberated from the complex. Sellers et al. [26] concluded that their enzyme latency was due to enzyme-inhibitor complex formation, but Vaes et al. [19] could not decide whether the latency of their collagenase and proteoglycanase activities was due to zymogen or enzyme-inhibitor complex.

As pointed out previously [7,14,27] changes in the chondrocyte microenvironment modulates the phenotypic expression of these cells. Although spinner-cultured chondrocytes appear to mimic more faithfully cartilage metabolism in vivo, most chondrocyte metabolic functions appear to be retained even when chondrocytes are cultured under monolayer conditions [28]. The culture environment may be the reason why monolayer culture produced more proteoglycanase and inhibitor than spinner culture. Changes in the cell-matrix environment may explain why more metal-dependent proteoglycanase activity was present in osteoarthritic rather than normal cartilage [22].

The neutral metal-dependent proteoglycanase, secreted by the chondrocytes in culture would presumably be capable of initiating as well as con-

tinuing destruction of the cartilage proteoglycan in joint disease from within the matrix. This enzyme activity may also be involved in physiological matrix turnover as well as being able to modify the matrix in the epiphyseal plate to permit calcification to occur [29]. Just as significant is the finding that the cultured chondrocytes release concurrently with the enzyme(s), inhibitory activity against proteoglycanase. Further study may elucidate the enzyme control mechanisms of articular cartilage and hopefully lead to development of therapeutic agents to retard the destruction of cartilage macromolecules in joint arthritides.

Acknowledgements

This work was supported in part by National Institutes of Health grants AM-08662 (D.S.H.), AG-02205 (C.J.M.), AM-16940, AG-01305 (R.W.M.) and the W.L. McKnight Arthritis Fund.

References

1 Howell, D.S., Sapolsky, A.I., Pita, J.C. and Woessner, J.F., Jr. (1976) Semin. Arthritis Rheum. 5, 365—383
2 Dingle, J.T. (1979) Ann. Rheum. Dis. 38, 201—214
3 Huybrechts-Godin, G. and Vaes, G. (1978) FEBS Lett. 91, 242—245
4 Hauser, P. and Vaes, G. (1978) Biochem. J. 172, 275—284
5 Janoff, A., Feinstein, G., Malemud, C.J. and Elias, J.M. (1976) J. Clin. Invest. 57, 615—624
6 Muir, H. (1977) Ann. Rheum. Dis. 36, 199—208
7 Sapolsky, A.I., Howell, D.S. and Woessner, J.F., Jr. (1974) J. Clin. Invest. 53, 1044—1053
8 Sapolsky, A.I., Keiser, H., Howell, D.S. and Woessner, J.F., Jr. (1976) J. Clin. Invest. 58, 1030—1041
9 Malemud, C.J., Weitzman, G.A., Norby, D.P., Sapolsky, A.I. and Howell, D.S. (1979) J. Lab. Clin. Med. 93, 1018—1030
10 Deshmukh-Phadke, K., Lawrence, M. and Nanda, S. (1978) Biochem. Biophys. Res. Commun. 85, 490—496
11 Fell, H.B. and Jubb, R. (1977) The destructive action of synovial tissue on articular cartilage in organ culture in: Rheumatoid Arthritis (Gordon, J.I. and Hazelman, B.I., eds.), pp. 192—197, Elsevier/North-Holland Biomedical Press, Amsterdam
12 Dingle, J.T., Saklatvala, J., Hembry, R., Tyler, J., Fell, H.B. and Jubb, R. (1979) Biochem. J. 184, 177—180
13 Sokoloff, L., Malemud, C.J. and Green, W.T. (1970) Arthritis Rheum. 13, 118—124
14 Norby, D.P., Malemud, C.J. and Sokoloff, L. (1977) Arthritis Rheum. 20, 709—716
15 Hascall, V.C. and Sajdera, S.W. (1969) J. Biol. Chem. 244, 2384—2396
16 Sapolsky, A.I., Altman, R.D., Woessner, J.F., Jr. and Howell, D.S. (1973) J. Clin. Invest. 52, 624—633
17 Malemud, C.J., Norby, D.P., Sapolsky, A.I. Matsuta, K., Howell, D.S. and Moskowitz, R.W. (1981) Biochim. Biophys. Acta 657, 517—529
18 Werb, Z., Dingle, J.T., Reynolds, J.J. and Barrett, A.J. (1978) Biochem. J. 172, 949—958
19 Vaes, G., Elkhout, Y., Lenaers-Claeys, G., Francois-Gillet, C. and Druets, J. (1978) Biochem. J. 172, 261—274
20 Sellers, A., Reynolds, J.J. and Merkle, M.C. (1978) Biochem. J. 171, 493—496
21 Sapolsky, A.I., Matsuta, K., Woessner, J.F., Jr. and Howell, D.S. (1978) Trans. Orthop. Res. Soc. 3, 71
22 Sapolsky, A.I., Matsuta, K., Howell, D.S. and Woessner, J.F., Jr. (1978) Arthritis Rheum. 21, 588
23 Roughley, P.J., Murphy, G. and Barrett, A.J. (1978) Biochem. J. 69, 721—724
24 Knight, J.A., Stephens, R.W., Bushell, G.R., Ghosh, P. and Taylor, T.K.F. (1979) Biochim. Biophys. Acta 584, 304—310
25 Kuettner, K.E., Croxen, R.L., Eisenstein, R. and Harper, E. (1976) Biochem. Biophys. Res. Commun. 72, 40—46
26 Sellers, A. and Reynolds, J.J. (1977) Biochem. J. 167, 353—360
27 Srivastava, V.M.L., Malemud, C.J. and Sokoloff, L. (1974) Connect. Tissue Res. 2, 127—136
28 Wiebkin, O.W. and Muir, H. (1977) J. Cell Sci. 27, 199—211
29 Blumenthal, N.C., Posner, A.S., Silverman, L.D. and Rosenberg, L.C. (1979) Calc. Tissue Int. 27, 75—82