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Original Contribution

HISTOCHEMICAL ANALYSIS OF THE ROLE OF CLASS I AND CLASS II *CLOSTRIDIUM HISTOLYTICUM* COLLAGENASE IN THE DEGRADATION OF RAT PANCREATIC EXTRACELLULAR MATRIX FOR ISLET ISOLATION

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Abstract — To understand why class II *Clostridium histolyticum* collagenase is much more effective than class I in the isolation of rat pancreatic islets, we analyzed the role of these collagenases in pancreatic tissue dissociation. Crude collagenase was purified and then fractionated into class I and II with different enzyme activities and protein compositions. Pancreatic tissue was incubated with either class I, class II, or class I + II, with or without added protease, under conditions that eliminated endogenous proteolytic activity. The degradation of pancreatic extracellular matrix was monitored by selective histochemical staining of tissue samples. Class I and II showed similar capacities to degrade glycoproteins and degraded about one-third of the glycoproteins during 120 min of incubation. The degradation of collagens by class I and II was relatively more effective, 80 to 95% of the collagens being removed in 120 min, and also class dependent. Both in the presence and absence of protease, class II was more effective at degrading collagens than class I, but this difference in efficacy was less apparent than with islet isolation. Class I + II degraded collagens faster and more complete than did the individual classes, indicating a synergistic effect of class I and II. Evaluation of collagen degradation at various pancreatic locations did not show a selective degradation of collagens by any of the collagenase classes. The present data offer a partial explanation for the major role of class II in islet isolation.
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Keywords — Class I & II collagenase; Rat pancreas; ECM degradation; Islet isolation.

INTRODUCTION

Isolation of islets of Langerhans requires selective dissociation of the pancreas, i.e., dissociation of the exo-

crine tissue without damaging the islets. Islet isolation is routinely performed by digestion of the pancreatic tissue with crude *Clostridium histolyticum* collagenase, a mixture of at least six different collagenases, neutral protease, other enzyme activities and unknown components. The considerable variation between different commercial collagenase preparations represents a major obstacle in the development of an efficient and reproducible islet isolation procedure and thus in making islet transplantation clinically applicable (8). With the aim of improving the efficacy of islet isolation procedures, we are studying the roles of individual enzymes in pancreas dissociation. Monitoring of the degradation of major extracellular matrix (ECM) components by histochemical methods (19) proved to be a useful tool for this purpose.

Collagenase plays a key role in pancreas dissociation because this enzyme, in the complete absence of other proteolytic activities, is capable of effectively degrading collagens in situ and, also, of removing the majority of proteoglycans and glycoproteins from the pancreatic tissue (19). Addition of a low concentration of *C. histolyticum* neutral protease enhances the degradation of all major ECM components and results in an improved tissue dissociation and islet release (19). Recently, we demonstrated that the two classes of *C. histolyticum* collagenase, class I and class II, have quite different roles in rat islet isolation (20). Class II collagenase is most effective in pancreas dissociation and islet isolation and class I plays a minor role, acting synergistically with class II. The present study was undertaken to try and find an explanation for this difference.

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The division of collagenases into two classes is based on their different substrate specificities as determined in vitro: class I collagenases have a high activity toward native collagen and a moderate activity toward the synthetic peptide 2-furanacryloyl-L-leucylglycyl-L-prolyl-L-alanine (FALGPA), while class II collagenases have a moderate activity toward native collagen and a high activity toward FALGPA (3). In addition, there are differences between the two classes with respect to their amino acid compositions, stabilities, responses to inhibitors, and active site metal substitutions, and pathways of collagen hydrolysis (1,2,4,5,11). The sequence specificities of class I and class II are similar but complementary, which accounts for their synergistic action in collagen digestion (17).

The capacities of the two collagenase classes to degrade various types of collagen have been studied by in vitro degradation experiments (5,10,18). In addition, we have studied the amount and distribution of total collagen (15) and of various types of collagen (12,13) in the rat pancreas. These studies require careful interpretation with respect to pancreas dissociation for islet isolation because the susceptibility to enzymatic degradation is quite different for collagens embedded in pancreatic tissue than for isolated collagens applied in vitro (18).

Therefore, we have purified class I and class II collagenase and studied their effects on pancreatic tissue directly by histochemical methods. The contributions of both classes to ECM degradation are interpreted in view of islet isolation.

MATERIALS AND METHODS

Materials

C. histolyticum collagenase (type XI, lot no. 69F-68051) was obtained from Sigma Chemical Co., St. Louis MO, and contained 4.8 U/mg FALGPA, 1920 U/mg collagen digestion, 20 U/mg neutral protease, and 1.1 U/mg clostripain according to the producer's specifications. Neutral protease was purified from crude *C. histolyticum* collagenase as described (19) and contained 950 U/mg solid caseinase activity.

Column materials were from Pharmacia (Uppsala, Sweden), Orange-collagen and Orange-gelatin were from BLOK Ltd (Vilnius, Lithuania), and gelatin was from Lamers-Indemans ('s Hertogenbosch, The Netherlands). All other materials have been described elsewhere (19,20).

Purification and Characterization of the Collagenase Classes

Commercial type XI collagenase was purified by gel filtration on a Sephacryl S-200 column (19,20), and stored freeze dried at -25°C . The activities of crude and

Table 1. Enzymatic activities of various collagenase preparations

Collagenase	Activity (U/mg Solid)		
	FALGPA	Orange-Collagen	Orange-Gelatin
Crude*‡	8.1	0.54	4.02
PNF†‡	12.2	0.64	6.91
Class I	3.4	1.14	8.66
Class I/II‡	6.1	0.14	4.13
Class II	29.1	0.06	0.58

*Crude = commercially available collagenase (Sigma type XI).

†PNF = purified nonfractionated collagenase; from this preparation the individual collagenase classes were purified.

‡Not used in histochemical analyses.

purified collagenase are given in Table 1. Purified collagenase (300 mg) was dissolved in 50 mL of 20 mM Tris-HCl, 1 mM CaCl_2 , pH = 7.5, and fractionated into class I and II collagenases by FPLC-anion exchange chromatography on a 50 mL HiLoad Q Sepharose column (6,20). Elution was performed with a linear gradient of 0–200 mM NaCl and the column was then rinsed with 1 M NaCl. Individual fractions were screened for their FALGPA hydrolytic and gelatinolytic activity and combined to three collagenase fractions. These were dialyzed against distilled water supplemented with 0.1 mM ZnCl_2 and 0.7 mM CaCl_2 to prevent irreversible activity loss during dialysis [Quick Info on Collagenase H of Boehringer-Mannheim, 1986; (4)] and stored freeze dried at -25°C . Purity and enzymatic activity were determined by 1) SDS-PAGE, 2) zymography on gelatin-containing SDS-gels, and 3) assays for FALGPA, collagenolytic, and gelatinolytic activity.

Pancreatic Tissue Dissociation and Histochemical Analysis

Tissue dissociation and the subsequent histochemical analysis were performed as described previously (19). Pancreata were removed from four male Wistar rats (350 g body weight) and distended with Krebs-Ringer-HEPES containing 10% (w/v) bovine serum albumin (KRH-BSA). The tissue was cut into small pieces (approximately $2.5 \times 2.5 \times 2.5$ mm) with a pair of scissors, washed three times, and divided into 11 aliquots of 2 g tissue suspension. Nine aliquots were transferred to 25 mL Erlenmeyer flasks for the histochemical analysis and two aliquots were used to determine the tissue wet weight: each aliquot of tissue suspension contained 0.35 ± 0.01 g pancreatic tissue. Trypsin inhibitors [2 mg/mL turkey egg white inhibitor and 3 mg/mL soybean inhibitor; (19)] were added and the final volume was adjusted to 10 mL/flask with KRH-BSA. Addition of BSA and trypsin inhibitors suppresses the endogenous proteolytic activity, which is required to analyze the individual

contributions of class I or II to ECM degradation, but it should be noted that these conditions also result in a reduced rate of dissociation as compared to routine islet isolations (19). Tissue dissociation was started by adding 2 mg of collagenase class I, or 2 mg of collagenase class II or a combination of 1 mg class I + 1 mg class II per flask, with or without 0.11 mg (100 U) of neutral protease. The flask was shaken at 37°C and 1.5 ml tissue samples were taken after 0, 15, 30, 60, 90, and 120 min. Tissue samples were washed three times with ice-cold KRH, fixed overnight in Bouin's solution, processed to paraffin sections, and then subjected to various histochemical stainings (19). Sections were stained with Sirius Red-Fast Green at pH 2 for collagens or with periodic-acid-Schiff (PAS) counterstained with Mayer's haemalum for glycoproteins. The amount of these ECM components was assessed by scoring the staining intensities in three sections of each sample blindly and calculating the mean. Staining intensities were scored on a scale of 0–4 [0 = complete absence of stain, 4 = staining intensity in native tissue at $t = 0$; (19)] by at least two independent observers. The total content of pancreatic collagens was calculated as the mean of the scores at four locations: in islets, around islets, around acini, and in septa. In addition, sections were stained with aldehyde fuchsin-azan (AF-azan) to assess the morphological quality and the degree of B-cell granulation of the islets, also scored on a scale of 0–4 (19).

SDS-PAGE and Zymography

Standard sodium dodecyl sulfate polyacryl amide gel electrophoresis (SDS-PAGE) was performed on gels containing 6.5% (w/v) acrylamide (19). Zymography was performed on SDS-gels containing copolymerized gelatin essentially as described by Heussen and Dowdle (7). Zymography gels contained 6.5% acrylamide at an increased crosslinking percentage, i.e., ratio bisacrylamide:acrylamide of 1:30 as compared to 0.8:30 in standard SDS-gels (7), and 0.1% (w/v) gelatin. Collagenases were dissolved in sample buffer containing 62.5 mM Tris-HCl, pH = 6.8, 2% (w/v) SDS, 10% (w/v) glycerol, 12.5 μ g/mL bromophenolblue, and 5 mM EDTA, and kept cool until electrophoresis. Electrophoresis was performed at about 15°C using water cooling. SDS was removed and enzyme activity restored by washing the gels in 2.5% (w/v) Triton X-100, 50 mM Tris-HCl, 5 mM EDTA, 0.02% (w/v) NaN_3 , pH = 7.5 at room temperature (2 washings of 10 min each). Gels were incubated for 60 min at 37°C in washing buffer containing 1% Triton X-100, briefly rinsed with water, and then stained with Coomassie brilliant blue (19). Gelatinolytic enzymes are detected as clear bands in a blue background.

Enzymatic Assays

Collagenase activity was measured by the FALGPA assay (16) and by the Orange-collagen and the Orange-gelatin assay (18,20). Activities are expressed as U/mg solid where 1 unit of enzyme activity is the amount of collagenase that hydrolyzes 1 nmol substrate per second. Neutral protease activity was measured as caseinase activity; 1 unit is defined as the amount of protease that hydrolyzes one mg of casein in 30 min (19).

Statistical Analysis

Data are presented as mean values $\pm$ SEM (standard error of the mean). Statistical analysis was performed using the nonparametric Friedman's test. Differences are considered to be statistically significant at $p < 0.05$ for comparisons at individual time points, and at $p < 0.01$ for comparisons between collagen degradation profiles (i.e., scores obtained after 15–120 min of tissue dissociation).

RESULTS

Purification and Characterization of Class I and II Collagenases

Purification of 1.5 g crude collagenase by gelfiltration chromatography yielded 0.7 g purified nonfractionated (PNF) collagenase with slightly higher enzymatic activities than crude collagenase (Table 1). Fractionation of PNF collagenase by ion exchange chromatography resulted in three peaks eluted by the salt gradient and a peak released by high salt (Fig. 1, panel A). Column fractions were assayed for their enzymatic activity using FALGPA and Orange-gelatin as substrates (Fig. 1, panel B). The first peak was high in FALGPA activity and low in gelatinolytic activity, which is characteristic for class II collagenases. The second peak had intermediate activities; it is indicated as class I/II because it was found that this fraction contained a mixture of class I and class II enzymes (see below). The third peak had a very high activity toward gelatin (and collagen; result not shown) and a low activity toward FALGPA and, therefore, contained class I collagenase. Peak fractions were pooled, dialyzed and freeze dried. From 300 mg of PNF collagenase we obtained 179.0 mg of class I (59.7%), 32.0 mg of class I/II (10.7%), and 43.5 mg of class II (14.5%), which indicates that this lot of Sigma type XI collagenase contains three to fourfold more class I than class II.

The specific enzymatic activities of the collagenase fractions were measured in three different assays (Table 1). Class I has a 19-fold higher activity toward collagen and a 15-fold higher activity toward gelatin than class II, and class II has an 8.6-fold higher FALGPA activity than

Fig. 1. Separation of purified collagenase into class I and II by anion exchange chromatography. Three hundred milligrams of PNF collagenase was applied to a 50 mL HiLoad Q Sepharose column and eluted with a linear 0–200 mM NaCl gradient. Panel A shows the protein profile (A280; solid line) and the NaCl profile (gradient followed by washing step with 1 M NaCl; broken line). Panel B shows the enzymatic activities of individual fractions toward FALGPA (▲) and Orange-gelatin (○). The gelatinolytic activities in fraction Nos. 49–55 are underestimated due to substrate limitation. The material eluted with 1 M NaCl contained little activity and was discarded. Fractions were pooled as follows: Nos. 34–36 = class II, Nos. 39–42 = class I/II, and Nos. 48–57 = class I.

class I. These activities are similar to those obtained in previous separations (18,20) and characteristic for the two distinct collagenase classes (4).

The composition of the classes was determined by SDS-PAGE (Fig. 2, left panel) and by zymography (Fig. 2, right panel). SDS-PAGE showed that each collagenase fraction contained a set of protein bands (two major and some minor) with mol.wts. between 68 and 115 kDa. All of these bands displayed gelatinolytic activity as detected by zymography (Fig. 2, right panel) and represent various collagenases. The presence of trace amounts of protein bands <60 kDa without gelatinolytic activity indicates that some spontaneous degradation occurred during the 6-month storage between performance of the

dissociation experiments with freshly purified class I and II and this SDS-PAGE run.

Although the protein compositions of class I and class II appear similar (Fig. 2, left panel), there are two observations that demonstrate that our class I and II fractions are composed of different collagenases. First, the two major bands in class II are much lower in gelatinolytic activity than those in class I (Fig. 2, compare left and right panel). Second, the major protein bands in class II are considerably less stable than those in class I as demonstrated by our finding that the bands in class II, but not those in class I, are subject to spontaneous degradation by standing overnight in 50 mM Tris-HCl, 5 mM EDTA, pH 6.8, at 4°C. The relative insta-

Fig. 2. SDS-gel electrophoresis and zymography of various collagenases. PNF collagenase and the separate collagenase classes (I, I/II, and II) were either denatured and subjected to standard SDS-PAGE (left) or run without previous denaturation on a gelatin-containing SDS gel (right). Both types of gel contain 6.5% acrylamide and were stained with Coomassie. Marker proteins are indicated on the left. The amounts of protein applied per lane were: 7.5 μ g PNF or 3.75 μ g purified class I, II, or I/II in SDS-PAGE and 30 ng of all collagenase preparations in zymography.

bility of class II concurs with the observation that this class is particularly sensitive to freeze-thawing and storage (4).

Our collagenase fractions were compared with the six collagenases purified and characterized in detail by Bond and van Wart (3,4) on the basis of their relative activities towards various substrates and the molecular weight, gelatinolytic activity and stability of the composing protein bands separated by SDS-PAGE. This allows the conclusion that our class II fraction contains two class II collagenases (ϵ = 110 kDa and most likely δ = 100 kDa). Our class I/II fraction contains the two smallest class I collagenases (α = 68 kDa and γ = 79 kDa) and a class II collagenase of about 110 kDa. Based on identical stability characteristics, this 110 kDa band can be identified as ϵ collagenase originating from the class II peak due to incomplete separation (see Fig. 1). Finally, our class I fraction contains the major class I collagenase β (115 kDa) and an unidentified collagenase of about 105 kDa.

Clearly, class I and class II represent completely different collagenase fractions, but class I/II contains a mixture of class I and class II enzymes. Therefore, class I/II was excluded from the histochemical analyses.

ECM Degradation by Different Enzymes

Pancreatic tissue was dissociated with 2 mg of collagenase class I, with 2 mg of collagenase class II, with 1

mg class I + 1 mg class II, or with 0.11 mg of neutral protease, always under conditions that suppress the endogenous proteolytic activity (19). The degradation of ECM components was monitored by selective histochemical staining of samples taken at regular intervals from the incubation vessels. The effects of various collagenase classes and of neutral protease on rat pancreatic tissue are shown in Fig. 3.

Collagens *in situ*—visualized by Sirius Red staining—are degraded at different rates by the various enzymes (Fig. 3A); the sequence in degradation rates is class I + II $\gg$ class II $>$ class I $>$ neutral protease $\gg$ no enzyme. Neutral protease has a lower collagen degradation capacity than the collagenases, while class II collagenase is significantly more effective in collagen degradation than class I. The collagen degradation rate by 1 mg class I + 1 mg class II is significantly higher than the degradation rates by 2 mg of either class I or class II, which indicates that the two classes act synergistically. Essentially all Sirius Red staining material has been removed from the tissue after 120 min of incubation with class I + II (Fig. 3A).

The degradation of glycoproteins—visualized by PAS staining—by various enzymes is shown in panel B of Fig. 3. Collagenase class I, class II, or neutral protease show little specificity with respect to the degradation of glycoproteins. All individual enzymes are capable to degrade about one-third of the glycoproteins present at t

Fig. 3. Degradation of collagen (A) and glycoproteins (B) by different enzymes. Pancreatic tissue (350 mg) was dissociated in 10 mL Krebs-Ringer-HEPES containing bovine serum albumin and trypsin inhibitors, and either 2 mg collagenase class I (○, $n = 4$), 2 mg collagenase class II (△, $n = 4$), 1 mg class I + 1 mg class II (□, $n = 4$), 0.11 mg (100 U) neutral protease (◇, dotted line; $n = 3$) or no enzyme (+, broken line; $n = 4$). Samples were processed for staining with Sirius Red (A) or periodic acid Schiff (B) and staining intensities were scored on a scale of 0–4. Values represent means $\pm$ SEM. Very small SEMs are not depicted.

= 0 during 120 min of incubation. Only with the combination class I + II a clear increase in the rate of glycoprotein degradation is seen, indicating a synergistic effect of class I and II in glycoprotein degradation (Fig. 3B).

Comparison of the degradation profiles of collagens and glycoproteins (Fig. 3) shows that 1) collagenases are more effective in collagen degradation than neutral protease, and 2) class I and class II collagenase show specificity in collagen degradation but not in glycoprotein degradation. These observations validate the selectivity of the histochemical staining methods.

ECM Degradation by Different Collagenases Combined With Neutral Protease

In islet isolation a low concentration of neutral protease is required to facilitate tissue dissociation (19,20).

Fig. 4. Degradation of collagen (A) and glycoproteins (B) by different collagenases combined with neutral protease. Pancreatic tissue (350 mg) was dissociated in 10 mL Krebs-Ringer-HEPES containing bovine serum albumin and trypsin inhibitors, and either 2 mg collagenase class I (●, $n = 4$), 2 mg collagenase class II (▲, $n = 4$), 1 mg class I + 1 mg class II (■, $n = 4$), or no collagenase (◇, dotted line; $n = 3$) in combination with 100 U of neutral protease. Samples were processed for staining with Sirius Red (A) or periodic acid Schiff (B) and staining intensities were scored on a scale of 0–4. Values represent means $\pm$ SEM. Very small SEMs are not depicted.

Therefore, we also performed dissociation experiments with class I and/or class II collagenase in combination with neutral protease (Fig. 4).

The presence of neutral protease resulted in a marked acceleration of the tissue dissociation process as indicated by visual inspection of the histological sections; this effect has been observed previously (19). Comparison of the collagen degradation patterns observed in the absence of protease (Fig. 3A) with those in the presence of protease (Fig. 4A) shows that protease enhances the degradation of collagen. When added to collagenase class I or class II, protease significantly increases the rate of collagen degradation and reduces the collagen content left after 120 min of incubation. Addition of protease to a combination of collagenase class I + class II also enhances collagen degradation significantly, albeit to a

Fig. 5. Collagen degradation at various locations in the pancreas. Pancreatic tissue (350 mg) was dissociated in 10 mL Krebs-Ringer-HEPES containing bovine serum albumin and trypsin inhibitors, and either 2 mg collagenase class I (●, $n = 4$), 2 mg collagenase class II (▲, $n = 4$), 1 mg class I + 1 mg class II (■, $n = 4$), or no collagenase (◇, dotted line; $n = 3$) in combination with 100 U of neutral protease. Samples were processed for staining with Sirius Red and staining intensities were scored in islets (A), around islets (B), around acini (C), and in pancreatic septa (D) using a scale of 0–4. Values represent means $\pm$ SEM. Very small SEMs are not depicted.

lesser extent than with the individual collagenase classes. A prominent effect of the addition of protease to collagenase class I + class II is the significant increase in the initial degradation rate of collagens (compare scores at $t = 15$ min in Figs. 3A and 4A). The amounts of undigested collagen at $t = 120$ min in the presence of protease are significantly lower after incubation with class II alone or with class I + II than after incubation with class I alone.

Neutral protease also enhances the degradation of glycoproteins (compare Figs. 3B and 4B). Glycoprotein degradation is equally effective by class I + protease, class II + protease or class I + class II + protease, and 40–50% of the PAS stain originally present in the tissue remains after 120 min of incubation with these enzymes (Fig. 4B). The difference in glycoprotein degradation rate between collagenase class I + II on the one hand and class I or class II on the other, as observed in dissociations without protease (Fig. 3B), is not observed in the

presence of protease (Fig. 4B). Our results suggest that glycoprotein degradation is a nonspecific process that is most effectively performed by a combination of at least two different proteolytic activities.

Collagen Degradation at Various Locations in the Pancreas

The selectivity of different enzymes in collagen degradation was assessed by scoring the staining intensities at various locations within the pancreas. Degradation of collagens by incubation of the tissue with neutral protease alone is very slow and incomplete at all pancreatic locations (Fig. 5). In the presence of one or two collagenase classes an adequate degradation of collagens is observed; the sequence in effectiveness is class I + class II > class II > class I at all four locations tested (Fig. 5). None of the classes shows a selective degradation of collagens at a distinct pancreatic location.

Fig. 6. Comparison of collagen degradation in the endocrine vs. the exocrine tissue. Pancreatic tissue (350 mg) was dissociated in 10 mL Krebs–Ringer–HEPES containing bovine serum albumin and trypsin inhibitors, and either 2 mg collagenase class I (○, ●), 2 mg collagenase class II (△, ▲), or 1 mg class I + 1 mg class II (□, ■) in combination with 100 U of neutral protease. Samples were processed for staining with Sirius Red and staining intensities were scored on a scale of 0–4 in the endocrine tissue (mean of scores in and around islets; open symbols and broken lines) and in the exocrine tissue (score around acini; closed symbols and solid lines). Values represent means $\pm$ SEM of four experiments. Very small SEMs are not depicted.

The time courses of collagen degradation in the exocrine tissue (score around acini; Fig. 5C) and in the endocrine tissue (mean of scores in and around islets; Figs. 5A and B) are compared in Fig. 6. The degradation of collagens in the exocrine tissue precedes the degradation of collagens in the endocrine tissue, both with class I and class II, but the differences are modest. With class I + II collagenase the difference between collagen degradation in the exocrine and the endocrine compartment is even smaller than with the individual classes (Fig. 6).

Quality of the Islets

The extent of islet damage during dissociation of pancreatic tissue with purified enzymes was determined by the morphological quality and the degree of B-cell granulation (i.e., the insulin content) of AF-azan stained islets. The morphological islet quality is nearly unaffected (score ≥ 3.8) during 120 min of dissociation with class I or class II collagenase. Comparison with the effects of PNF collagenase on islet quality (19) also shows that the purified classes are relatively mild to islets. The insulin content is also high (score ≥ 3.2) when dissociation is performed with collagenase class I, II, or I + II, in the absence as well as in the presence of protease. Only when the tissue is incubated with protease alone, some reduction in B-cell granulation is observed.

DISCUSSION

Theoretically, a collagenase effective in islet isolation would act selectively by degrading the collagens in the exocrine tissue while leaving those in and around the islets intact (14). That this theoretical approach may actually be practically applicable is corroborated by our previous observations that the combination of a low amount of collagen between the acini and a relatively high amount of collagen in and around the islets is favorable for effective pancreas dissociation (15), and that class I and class II have different roles in islet isolation (20). Our present histochemical analysis offers new information on the role of individual enzymes in the degradation of pancreatic ECM components but, somewhat disappointingly, we found no selective degradation of collagens by either class I or class II.

Collagens at all pancreatic locations are degraded faster by class II than by class I. One explanation for the relatively high rate of collagen degradation by class II may be found in the broader sequence specificity of class II as compared to class I (17). This suggests that class II is better equipped to degrade those collagens which are protected against enzymatic attack by coating with proteoglycans (9) or perhaps other ECM components. A second explanation can be found in the specificities of the collagenase classes for various types of collagen. Using immunohistochemical methods, we have demonstrated that the rat pancreas contains collagen types I, III, and V, and that these types of collagen are colocalized in the acinar septa and in the interface between acini and islets (13). Mallya et al.'s demonstration that, in vitro, fibrillar collagen of types I and III are more readily digested by ϵ -collagenase (a major component of our class II) than by β -collagenase (a major component of our class I) (10) therefore explains the observed difference between class I and II in collagen degradation. The validity of both explanations, however, is not certain because the enzymatic hydrolysis of collagens embedded in pancreatic tissue differs from that of isolated collagens (18,20). This phenomenon is also demonstrated in the present study: class I has the highest collagen digestion activity in vitro (Table 1) but class II shows a higher degradation rate of pancreatic collagens (Fig. 3A).

The two classes of collagenase act synergistically in the degradation of collagen, because degradation by a combination of 1 mg of an effective collagenase (class II) + 1 mg of a less effective collagenase (class I) is faster and more complete than degradation by 2 mg of class II (Figs. 3A and 4A). A synergistic effect of class I and II was also observed in pancreas dissociation and islet isolation (20); it can be ascribed to the similar but complementary sequence specificities of the two classes (17).

Neutral protease increases the rates of collagen degradation by both class I and class II collagenase but the difference between the two classes remains (Fig. 4A). The observation that class II + protease is more effective in collagen degradation than class I + protease corroborates our previous finding that, in the presence of protease, class II collagenase is much more effective in pancreas dissociation and islet isolation than class I collagenase (20). The difference between the two classes in collagen degradation is, however, much smaller than expected on the basis of their markedly different efficacies in islet isolation. For example, 1 mg of class II gives an adequate tissue dissociation and a high islet yield as compared to 2 mg of class I (20), while collagen degradation with 2 mg of class II is only slightly faster than that with 2 mg of class I (Fig. 4A). Another discrepancy concerns our finding that 2 mg of class I is ineffective in pancreas dissociation and islet isolation (20), whereas the same amount of class I, albeit under slightly different experimental conditions [(20); and Materials and Methods], is well capable to degrade most of the pancreatic collagens (Fig. 4A). A similar phenomenon was observed in dissociations with PNF collagenase without any protease: under these conditions a slow and incomplete dissociation occurred in spite of the fact that the large majority of collagens were degraded (19).

Our results imply that an effective digestion of collagens does not guarantee tissue dissociation and that the disruption of other tissue maintaining structures, possibly cell-to-cell and cell-to-matrix contacts (12), may be an additional requirement for the dissociation of pancreatic tissue. They also show that there is no strict correlation between the collagen degradation capacity of an enzyme and its capacity to dissociate pancreatic tissue. This indicates that histochemical analysis alone is not a reliable parameter to predict the usefulness of an enzyme in islet isolation.

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