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Prolyl Endopeptidase Purified From Granulomatous Inflammation in Mice

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Abstract Activity of prolyl endopeptidase (EC 3.4.21.26) which hydrolyses the Pro^7-Phe^8 bond in angiotensin II has been found to elevate in experimentally produced granulomatous inflammation in liver and skin. We purified the enzyme 1,536-fold by 6 steps from murine hepatic granulomas. The purified enzyme has a molecular weight of 79 kDa and physicochemical properties equivalent to those previously reported for prolyl endopeptidase purified from other sources. By HPLC analysis, the cleavage of Phe^8-Leu^{10} and Phe^8 from angiotensin I and II, respectively, was detected and quantified. Monospecific IgG was prepared from serum of rabbits injected with purified enzyme. Concentration of the enzyme was immunohistochemically detected in cells which form granulomatous organization, but not in inflammatory cells surrounding the foci. The antibody, however, cross reacted with the enzyme in adjacent liver cells and weakly stained their cytoplasm. The findings indicate that this enzyme, in addition to angiotensin converting enzyme, may serve as a useful biochemical marker for granulomatous tissue reactions. © 1992 Wiley-Liss, Inc.

Key words: prolyl endopeptidase, granulomatous tissue reaction, angiotensin system, hydrolysis of angiotensin I and II, purification and characterization, immunohistochemistry

Granulomatous tissue reaction is a chronic condition characterized by accumulation and organization of activated macrophages, and formation of epithelioid cells [1–3]. Although exact pathomechanisms which result in the disease process are unknown, angiotensin II, a potent vasoactive peptide, has been shown to alter macrophage function [e.g., 4,5] through angiotensin II receptors on the cells [5]. The activity of angiotensin converting enzyme (ACE) which hydrolyses angiotensin I to angiotensin II is elevated in serum of patients with sarcoidosis [6], a disease characterized by organized granulomas. Furthermore, ACE activity has been shown to increase in liver [7,8] and skin [9] with enlargement of the granulomatous tissue reaction, suggesting a direct correlation between angiotensin II formation and activity of the disease process. Prolyl endopeptidase is a hydrolase for angiotensin II [10,11], and it also is expected to release Phe^8-Leu^{10} from angiotensin I. We measured the enzyme activity in experimentally produced murine granulomas. As suspected, the activity was much greater in both hepatic and skin granulomas than normal liver and skin [12], indicating that this enzyme in addition to ACE, may be involved in regulation of the granulomatous tissue reaction. In the present study we purified prolyl endopeptidase from murine hepatic granulomas to apparent homogeneity. The purified enzyme was biochemically characterized and hydrolysis of angiotensin I and II determined. In addition, we immunohistochemically demonstrated that reactivity of monospecific antibody to the enzyme is accentuated in granulomatous tissue compared with adjacent hepatic cells.

Abbreviations used: -2-NNap, β-naphthylamide; SDS, sodium dodecyl sulfate; Z-Pro-prolinal, N-benzyloxycarbonyl-L-prolyl-L-prolinal; HPLC, high-performance liquid chromatography; PCMB, *p*-chloromercuribenzoic acid; Z-Gly-Pro-MCA, N-benzyloxycarbonyl-glycyl-L-proline-4-methyl-coumaryl-7-amide; AMC, 7-amino-4-methyl-coumarin; DFP, diisopropyl fluorophosphate.

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MATERIALS AND METHODS

Infection and Isolation of Granulomas

Female C57BL/6 strain mice (5–6 weeks old) were purchased from Simonsen Laboratories (Gilroy, CA). The mice were injected subcutaneously with 75 cercariae of the Puerto Rican strain of *Schistosoma mansoni*. At 9 weeks after infection, the mice were killed and their livers

were removed. The granuloma-rich fraction was isolated from other hepatic cells by homogenization in phosphate buffered saline solution in a Waring blender, and subsequent filtration through a stainless steel mesh. Livers from age-matched noninfected mice were taken for a control.

Chemicals

Z-Gly-Pro-MCA and all 2-NNap peptide derivatives were obtained from BACHEM Bioscience (Philadelphia, PA), and angiotensin I and II were purchased from STAR Biochemicals (Torrance, CA). The specific inhibitor of prolyl endopeptidase, Z-Pro-prolinal, was kindly provided by Dr. Tadashi Yoshimoto, Nagasaki University (Japan).

Assay for Prolyl Endopeptidase Activity

The method of Yoshimoto et al. [13] was used for hydrolysis of Z-Gly-Pro-MCA as a substrate. To 0.25 ml of 0.2 M phosphate buffer (pH 7.0), was added 0.2 ml of enzyme solution, and the mixture was pre-incubated at 37°C for 3 min. The reaction was initiated by adding 50 μl of 1 mM Z-Gly-Pro-MCA in 40% dimethylsulfoxide. After incubating at 37°C for 10 min, the reaction was stopped by adding 0.5 ml of 0.7 M acetate buffer (pH 4.0). The fluorescence of AMC was measured at 370 nm of excitation and 440 nm of emission. One unit of the enzyme activity was defined as the amount of the enzyme that released 1 μmol of AMC per min at 37°C. Protein concentration was determined by the Lowry method [14], using bovine serum albumin as the standard.

Purification of Prolyl Endopeptidase

Hepatic granulomas were homogenized in 20 mM Tris-HCl buffer (pH 7.0) containing 10 mM EDTA and 10 mM 2-mercaptoethanol in a glass homogenizer. The homogenate was centrifuged at 10,000*g* for 20 min, and the supernatant fractionated by ammonium sulfate precipitation at 50% to 80% saturation. The resultant precipitate was dissolved in a small volume of 20 mM Tris-HCl buffer (pH 7.0) containing 10 mM 2-mercaptoethanol, desalted by gel filtration on a Sephadex G-25 column (2.6 × 58 cm) and then applied to a Q Sepharose Fast Flow column (2.4 × 10 cm) equilibrated with the same buffer. The adsorbed enzyme was eluted with a linear gradient (0–0.5 M) of NaCl. The active fractions were combined, concentrated with YM-10 Diaflo membrane (Amicon) and applied to a Sephacryl S-200 column (1.6 × 114 cm) equilibrated with 20 mM Tris-HCl buffer (pH 7.0) containing 10 mM EDTA, 10 mM 2-mercaptoethanol, and 0.1 M NaCl. The active fractions were combined and the enzyme was precipitated by 80% saturation of ammonium sulfate. It was dissolved in a small volume of 5 mM phosphate buffer (pH 7.0) containing 10 mM 2-mercaptoethanol, and desalted with a Sephadex G-25 column. It was applied to a column (2 × 6 cm) of hydroxyapatite and the adsorbed enzyme was eluted with a linear gradient (5–500 mM) of potassium phosphated buffer (pH 7.0). The active fraction was combined, concentrated with a YM-10 membrane, and the buffer was changed to 20 mM Tris-HCl (pH 7.0) containing 10 mM EDTA. The enzyme solution was then applied to a PCMB-Sepharose column (1 × 12 cm), prepared by the method of Yokosawa et al. [15] but using EAH-Sepharose 4B (Pharmacia) for coupling. The adsorbed enzyme was eluted with 20 mM Tris-HCl buffer (pH 7.0) containing 20 mM 2-mercaptoethanol and 0.5 M NaCl after washing the column with 20 mM Tris-HCl buffer (pH 7.0) containing 10 mM EDTA and 0.1 M NaCl. The combined active fraction was concentrated with Centricon 10 (Amicon), applied to a TSK-250 column (0.75 × 60 cm, Bio-Rad) and eluted on an HPLC system (Shimadzu Scientific Instruments) at a flow rate of 0.5 ml per min. The buffer system used was 20 mM Tris-HCl (pH 7.0) containing 10 mM EDTA, 10 mM 2-mercaptoethanol, and 0.1 M NaCl.

Enzymatic Properties

Optimum pH and pH stability were determined in buffers; 0.1 M acetate (pH 3.5–5.5), 0.1 M phosphate (pH 6.0–7.25), 0.1 M Tris-HCl (pH 7.5–8.5), and 0.1 M borate-NaOH (pH 9.0–11.0). Optimum temperature was tested in 0.1 M phosphate buffer (pH 7.0) at 25–60°C for 10 min. Molecular weight was estimated by HPLC using a TSK-250 column (0.75 × 60 cm) calibrated with gel filtration standard (Bio-Rad). The buffer system used was 50 mM phosphate (pH 7.0) containing 0.2 M NaCl at a flow rate of 0.5 ml per min at 25°C. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli [16] was also used for estimation of the molecular weight. An electrophoresis calibration kit (Pharmacia) was used for the standard protein markers. Isoelectric point was determined by the method of Vesterberg and Svensson [17]

using ampholyte in the pH range of 3.5–10.0 with a sucrose density gradient of 0% to 50%. Focusing conditions were 400V at 4°C for 40 h.

Effects of Inhibitors

An aliquot of enzyme preparation dialyzed against 20 mM Tris-HCl buffer (pH 7.0) was pre-incubated with the inhibitor for 10 min at 37°C and the residual activity for Z-Gly-Pro-MCA was measured under standard conditions.

Substrate Specificity

Hydrolysis of a 2-NNap peptide derivatives was assayed with 0.54 μg of the purified enzyme at 37°C in 0.1 M phosphate buffer (pH 7.0) for various lengths of time. Initial velocity of hydrolysis at various substrate concentrations was determined by measuring the amount of released 2-naphthylamine, as detected by colormetric reaction with *p*-dimethylaminocinnamaldehyde by the method of Ito et al. [18]. K_m and V_{max} values of the enzyme were determined from Lineweaver-Burk plot. The k_{cat} values were calculated on the basis of a molecular weight of 79 kDa.

Hydrolysis of Angiotensin I and II

Angiotensin I (193 μM) and II (239 μM) were incubated with 0.54 μg of the enzyme preparation in 0.1 M phosphate buffer (pH 7.0) in a final volume of 200 μl for 0–60 min. The reaction was stopped by the addition of 10 μl 6 N HCl. The digest (20 μl) was applied to a TSK Gel ODS-120T column (4.6 × 250 mm, Toyo Soda), and eluted with a 15 min linear gradient (20–28%) of acetonitrile containing 0.05% trifluoroacetic acid at a flow rate of 1 ml/min at 25°C. Absorbance at 210 nm was monitored. The retention times for angiotensin I and II (0 min digest), and the products which appeared after digestion were recorded, and amino acid composition of the all peaks were determined. The concentration of angiotensin I and II and the fragments produced were calculated from the peak areas of standard angiotensin I and II. Kinetic parameters were calculated as described for synthetic substrates.

Immunohistochemistry

Antiserum to prolyl endopeptidase purified from hepatic granulomas was produced in rabbits and monospecific IgG was purified by affinity chromatography using a PSE-coupled sepharose 4B column [19]. Immunohistochemial staining was performed by the avidin-biotin complex method using formalin fixed and paraffin embedded tissue [20].

RESULTS

Comparison of Activity in Normal and Granulomatous Tissues

Z-Gly-Pro-MCA hydrolase activity was 8.4 ± 0.9 mU/mg protein of crude extract prepared from hepatic granulomas, while the activity was about 3.7 fold increase over the activity from normal liver extract (2.3 ± 0.3 mU/mg protein).

Purification of Prolyl Endopeptidase From Hepatic Granulomas

Total and specific activity calculated at each of 6 purification steps from crude extract are summarized in Table I. Q Sepharose Fast Flow column chromatography used instead of the authentic anion exchange chromatography [13,21], was found to accommodate a speedy separation with comparable results (Fig. 1). The enzymatically active fractions, pooled and applied to a Sephacryl S-200 column, eluted at a molecular weight of approximately 80 kDa (between 160

TABLE I. Purification of Prolyl Endopeptidase From Hepatic Granulomas*

Purification step	Protein (mg)	Total activity[a] (units)	Yield (%)	Specific activity (units/mg)	Purification (fold)
Supernatant of homogenate	4,001	14.4	100	0.0036	1
Ammonium sulfate fractionation	212	4.02	27	0.019	5
Q Sepharose	34.1	3.47	24	0.102	28
Sephacryl S-200	6.3	2.48	17	0.394	109
Hydroxyapatite	0.78	1.41	10	1.81	502
PCMB-Sepharose	0.34	1.24	9	3.65	1,014
HPLC (TSK-250)	0.17	0.94	7	5.53	1,536

*The values given are from a single preparation of 33 g of hepatic granuloma.
[a]Enzyme activity was monitored during purification of prolyl endopeptidase using Z-gly-pro-MCA as the substrate.

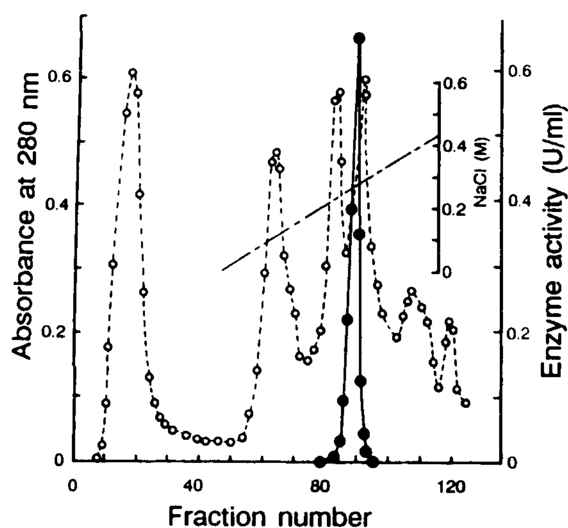

Fig. 1. Q Sepharose Fast Flow chromatography of partially purified prolyl endopeptidase from hepatic granulomas. Fractions of 5.0 ml each were collected at a flow rate 150 ml/h. NaCl gradient (---); Z-Gly-Pro-MCA hydrolytic activity (●); protein (○) measured at 280 nm; Active fractions (86 to 92) were collected for further purification.

kDa of γ-globulin and 45 kDa ovalbumin used for calibration). Following sequential hydroxyapatite and PCMB-Sepharose column chromatography, the active fraction was recovered as a single peak at approximately 0.1 M potassium phosphate buffer in step one, and with the elution buffer containing 20 mM 2-mercaptoethanol in the second step. The enzyme finally was purified to 1,547-fold by HPLC gel filtration, and was used for characterization. This enzyme source was identified as a single band on SDS-PAGE (Fig. 2). We used the identical steps for purification of Z-Gly-Pro-MCA hydrolase from normal mouse liver. After the hydroxyapatite column chromatography, the normal liver enzyme showed 0.542 U/mg protein (361-fold purification).

Enzymatic Properties

Physicochemical properties of prolyl endopeptidase purified from hepatic granulomas of mice were determined using Z-Gly-Pro-MCA as a substrate (Table II). They were equivalent to those reported for the enzyme purified from other mammalian cell sources [e.g., 13,22,23]. The optimum pH of the enzyme was 7.0. The enzyme retained more than 70% of the original activity between pH 5.5 and 7.5 after incubation at 37°C for 1 h. The optimum temperature for activity was 41°C for a 10 min enzyme reaction. The

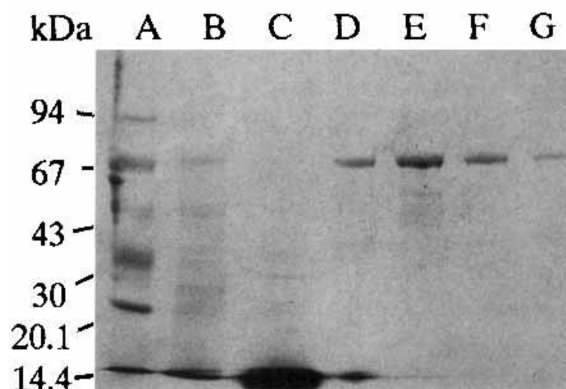

Fig. 2. SDS-polyacrylamide gel electrophoresis analysis of prolyl endopeptidase from hepatic granulomas. Proteins from each purification step were applied to a 9% slab gel and were stained with Coomassie brilliant blue R250: marker proteins (**lane A**); proteins after ammonium sulfate fractionation (**lane B**); and Q Sepharose (**lane C**); Sephacryl S-200 (**lane E**) and PCMB-Sepharose 4B (**lane F**) column chromatography. The purified enzyme after HPLC gel filtration (**lane G**) shows a single protein band.

molecular weight of the purified enzyme was estimated to be 79 kDa and 78 kDa by HPLC gel filtration and by SDS-PAGE, respectively. The isoelectric point was pH 4.75.

Effect of Chemical Reagents, Metal Ions, and Inhibitors

The enzyme was markedly inhibited by DFP, PCMB, N-ethylmaleimide, Zn^{2+}, Cu^{2+}, and Hg^{2+} (Table III). A synthetic inhibitor specific for prolyl endopeptidase [24,25], Z-Pro-prolinal completely inhibited the enzyme at a concentration of 1 μM. The activity was not inhibited by E-64, Mn^{2+}, and soybean trypsin inhibitor, or metal chelators such as EDTA.

TABLE II. Enzymatic Properties of Prolyl Endopeptidase From Hepatic Granuloma*

	Hepatic granuloma
Optimum pH	7.0
pH stability[a]	5.5–7.5
Optimum temperature[b]	41°C
Molecular weight	
By gel filtration	79 kDa
SDS-PAGE	80 kDa
Isoelectric point	4.75

*Standard substrate used was Z-gly-pro-MCA.
[a]70% remaining activity after preincubation at 37°C for 60 mins.
[b]At pH 7.0.

TABLE III. Effect of Chemicals and Metal Ions on Prolyl Endopeptidase From Hepatic Granulomas

Chemicals	Concentration (mM)	Remaining activity[a] (%)
None	—	100
EDTA	1.0	100
PCMB	0.1	3
Iodoacetate	1.0	85
N-ethylmaleimide	0.1	3
E-64	0.1	100
DFP	1.0	3
PMSF	1.0	13
Chymostatin	1.0	18
Soybean trypsin inhibitor	0.1	100
Tos-Lys-CH_2Cl (TLCK)	1.0	26
Z-Pro-prolinal	0.001	0
Zn^{2+}	1.0	4
Mn^{2+}	1.0	100
Cu^{2+}	1.0	2
Fe^{2+}	1.0	97
Hg^{2+}	1.0	2

[a]The enzyme (1.1 units) was incubated in 0.1 M phosphate buffer (pH 7.0) for 10 min at 37°C with the chemical. The residual activity was assayed by the standard method and the activity without the modifier is taken as 100%.

Substrate Specificity

As summarized in Table IV, the enzyme was completely inert toward Pro-2-NNap, Z-Pro-2-NNap, and Gly-Pro-2-NNap. When the chain length was elongated at the amino terminal, the peptides such as Z-Gly-Pro-2-NNap became susceptible to the enzyme. Z-Gly-Pro-MCA showed lower K_m value and higher k_{cat} than the other substrates, thus it obtained the highest k_{cat}/K_m, and was the substrate with the greatest affinity for the enzyme.

Hydrolysis of Angiotensin I and II

Without the enzyme digestion both angiotensin I (Fig. 3A, peak 1) and angiotensin II (Fig. 3D, peak 1) appeared as a single peak with retention time of 27.5 min and 25.4 min, respectively. After 10 min of incubation with prolyl endopeptidase, reduction of the peak 1 and newly appearance of 2 additional peaks were detected. Amino acid analysis showed that peak 2 (retention time 24.1 min) was Phe-His-Leu and peak 3 (retention time 19.8 min) was Asp-Arg-Val-Tyr-Ile-His-Pro from angiotensin I (Fig. 3B), and Asp-Arg-Val-Tyr-Ile-His-Pro (peak 2, retention time 19.8 min) and Phe (peak 3, retention time 9.3 min) from angiotensin II (Fig. 3E). As shown in Figure 3C and 3F, prolongation of enzyme digestion up to 30 min further decreased the amounts of angiotensin I and II, as well as increased the peak areas of their products. Formation of stoichiometric amounts of all fragments was confirmed by quantification of angiotensin I and II, as well as their fragments during 5 to 60 min of incubation time. Hydrolysis rates of the two natural substrates showed that the k_{cat}/K_m value to be lower than Z-Gly-Pro-MCA and Z-Ala-Pro-2-NNap (Table IV).

Immunohistochemical Detection of Prolyl Endopeptidase in Granulomatous Inflammation

Granulomatous tissue reaction consisting primarily of activated macrophages, epithelioid cells and other inflammatory cells developed in mouse liver 9 wks after *S. mansoni* infection (Fig. 4A). The reaction product with anti-prolyl endopepti-

TABLE IV. Substrate Specificity of Prolyl Endopeptidase From Hepatic Granuloma*

Substrate	S_o (mM)	K_m (mM)	$[E_o]$ ($\times 10^{-8}$ M)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1} s^{-1}$)
Synthetic substrate					
Pro-2-NNap			not hydrolyzed		
Z-Pro-2-NNap			not hydrolyzed		
Gly-Pro-2-NNap			not hydrolyzed		
Z-Gly-Pro-MCA	0.008–0.1	0.015	1.2	10.53	702
Z-Gly-Pro-2-NNap	0.008–0.1	0.029	1.2	1.57	54
Z-Ala-Pro-2-NNap	0.008–0.1	0.018	1.2	4.46	247
Native substrate					
Angiotensin I (human)	0.009–0.05	0.016	1.2	1.12	70
Angiotensin II (human)	0.009–0.05	0.036	1.2	0.57	15

*K_m and V_{max} were determined by Lineweaver-Burk plot; and k_{cat} values for prolyl endopeptidase were calculated on the basis of molecular weight 79 kDa.

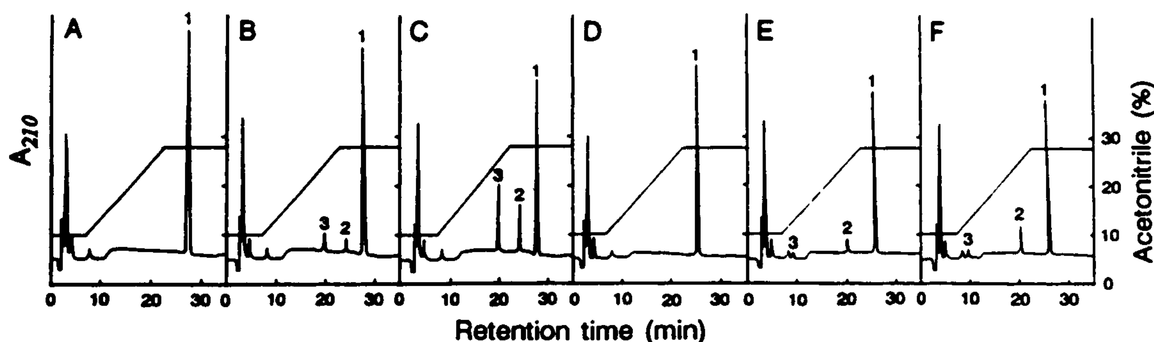

Fig. 3. HPLC identification of the hydrolytic products from angiotensin I (A, B, C) and II (D, E, F) by prolyl endopeptidase. Incubation times were 0 min (no incubation: A, D), 10 min (B, E), 30 min (C, F). Retention time (Rt) of angiotensin I and II standard was 27.5 min (peak 1 in A, B, C) and 25.4 min (peak 1 in D, E, F), respectively. Products detected after 10 or 30 min incubation were Asp^1-Pro^7 (peak 3 in B, C; peak 2 in E, F, Rt = 19.8 min), Phe^8-Leu^{10} (peak 2 in B, C, Rt = 24.1 min), and Phe (peak 3 in E, F, Rt = 9.3 min).

dase IgG was detected in the cytoplasm of centrally located cells which form the granulomatous organization around parasite eggs, but not in inflammatory cells that accumulated in the peripheral area (Fig. 4B,C). Some hepatic cells also showed weak reactivity and Western blot analysis identified that molecular weight of the antigens is identical in both granulomas and normal liver, and 79 kDa (data not shown).

DISCUSSION

Normal tissue distribution studies of Z-Gly-Pro-2-NNap hydrolyzing enzyme in rat [13] and rabbit [26] have indicated that the liver is one of tissues with higher activity. In this study further increase of the enzyme activity was detected in the granulomatous tissues which developed in mouse liver. Properties of the enzyme purified from hepatic granulomas are comparable to prolyl endopeptidases purified from other mammalian tissues [23,26] and plants [27,28]. The enzyme is stable at neutral pH and optimal activity is detected at neutral pH. The inhibitor profile also was characteristic for prolyl endopeptidase [21,26,29], and participation of both seryl and cysteinyl residues in the catalytic action was

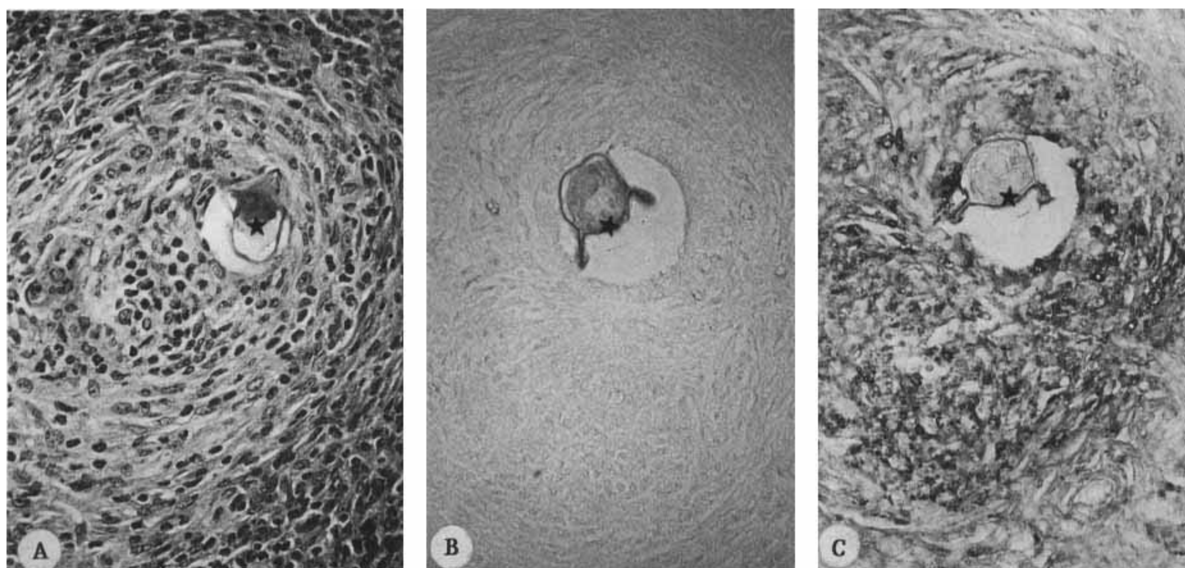

Fig. 4. Immunohistochemical detection of prolyl endopeptidase in granulomatous inflammation which develop in mouse liver by 9 weeks after *Schistosoma mansoni* infection. Hematoxylin and eosin stained (A) paraffin section shows two types (central and organized vs. peripheral and diffuse) of cellular distribution. Reactions with pre-immune rabbit IgG (B), and monospecific antibody (C). *Parasite egg; Magnification ×650.

demonstrated. The catalytic activity of the granuloma enzyme against synthetic substrates was almost identical to previously reported findings [26,29], and we have then added kinetic data of the enzyme activity on the cleavage of Pro^7-Phe^8 from angiotensin I and II.

Polyclonal antibody generated in rabbits showed cross reactivity between the enzymes from normal liver and granulomas. However, the immunohistochemical reaction of the antibody over hepatic cells was weak, compared with that in the granulomatous area; clearly, the enzyme concentrated within granulomas, supporting the view that the cells responsible for the increased enzyme activity in granulomas are activated macrophages and epithelioid cells. In skin, prolyl endopeptidase activity was shown to correspond well with the elevation of ACE activity and the degree of granulomatous tissue reaction [12], indicating that this enzyme also may serve as a biochemical marker for granulomatous tissue reaction. Furthermore, these findings suggest that prolyl endopeptidase in hepatic granulomas also may be involved in regulation of the angiotensin system in granulomatous tissue reactions. Elevation of ACE activity [6–9] and detection of the enzyme [30] and angiotensin I and II [4,5] in macrophages which compose granulomas have been known for some time, although how the angiotensin system modulates this unique tissue reaction remains unclear. On the other hand, information has accumulated to show that angiotensin II is not the only active angiotensin peptide in cardiovascular regulation and fluid homeostasis. Angiotensin II is a potent inhibitor of adenylate cyclase [31,32] and increases cytosolic calcium through an inositol triphosphate mediated mechanism [33,34]. The presence of angiotensin II specific receptors has been demonstrated in guinea pig peritoneal exudate [35,36] and human mixed mononuclear cells [37,38], in addition to mouse hepatic granuloma cells [5], indicating that the known biological functions of angiotensin II may be directly involved in granulomatous tissue responses. From the pattern and level of elevated prolyl endopeptidase activity found in this study, we speculate that the enzyme is functional in the angiotensin system which is operative in granulomatous tissue reactions. At least two possibilities exist. First, the endopeptidase may maintain homeostasis of the system by hydrolyzing both angiotensin I and II, while ACE is generating angiotensin II from angiotensin I. Since the endopeptidase hydrolyses angiotensin I most effectively, this action would serve to reduce the intercellular effect of ACE. The time course study in the skin granuloma model [12], which shows a parallel elevation of the two enzymes, strongly supports such a relationship. Alternatively, prolyl endopeptidase may process a bioactive peptide from angiotensin II in macrophages. This notion comes from the observation of Simon et al. [39] that angiotensin II is hydrolyzed into tyrosine containing small peptides prior to demonstrable biological action. If this possibility is confirmed by further experiment, it would imply almost the opposite effect as concerns prolyl endopeptidase modulation of the granulomatous tissue reaction. This alternative proposal suggests the total amount of "bioactive peptide" would increase beyond that produced by ACE alone, because prolyl endopeptidase could form a biologically active, tyrosine containing peptide from both angiotensin I and II, and up-regulate the tissue reaction probably much more effectively than ACE.

The most simplistic view that formation of angiotensin II underlies the disease process was denied in earlier experiments, where use of the ACE inhibitor, captopril, not only failed to control but aggravated granuloma formation [40,41]. Administration of inhibitors, such as thiazolidine derivatives [25] and/or anti-immunoglobulin, to prolyl endopeptidase to mice undergoing a granulomatous tissue reaction could be used to further explore the relationship of the enzyme to the angiotensin system and the granulomatous response. Theoretically, both methodologies are available, although practical usage remains to be proven. Animal experiments must be formulated carefully, since use of these inhibitors could lead to an effective therapy of patients with disabling granulomatous disease.

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