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Fibrinolytic System in the Ascites of Experimental Pancreatitis

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ABSTRACT

Fibrinolytic enzymes in the ascites were studied in experimental acute pancreatitis induced in rats. Among the amidolytic activities towards synthetic substrates, that towards pyro-Glu-Gly-Arg-pNA・HCl was the highest, followed by H-D-Ile-Pro-Arg-pNA・2HCl. Fibrinolytic activity was observed in 8 of 18 rats. The main fibrinolytic enzyme had a molecular weight of about 50,000 by zymography. Of the fractions separated from the ascites by Lys-Sepharose affinity chromatograohy, the nonadsorption fraction revealed a lytic zone in the 50,000-80,000 molecular weight range and the fraction eluted with 0.6M NaCl was observed mainly at the 100,000 molecular weight position on zymohraphy. After concentration, these fractions exhibited a lytic zone at the same position of molecular weight 50,000, which was not detected using plasminogen-poor fibrin agar plates. The activity of the NaCl elution fraction was increased by trypsin treatment. When incubated homogenate of pancreas from healthy rats was injected intraperitoneally into healthy rats, the recovered fluid displayed a zymographic pattern which consisted of such various lytic zones as were observed for the ascites of pancreatitis. These findings suggest that part of the increase of fibrinolytic activity in the ascites during pancreatitis was attributable to the release of precursor of plasminogen activator and its conversion to the active form by intrapancreatic substances.

INTRODUCTION

It has been reported that substances which cause deterioration of systemic manifestations to occur are present in the ascites during human or experimental acute pancreatitis [1-4] The availability of peritoneal lavage as a treatment for pancreatitis [5,6] is interpreted to confirm that such substances can certainly exert systemic effects during the course of pancreatitis. Concerning the presence of substances related to the coagulation and fibrinolytic systems in the ascites, Satake et al. [7] demonstrated this by the intravenous injection of ascites of experimental pancreatitis into healthy animals. It has also been reported that the proteases such as trypsin, elastase and plasma kallikrein are present in the ascites. [8-10] Such proteases originally have the property of influencing the coagulation and fibrinolytic system, [11-14] and the circulating pancreatic proteases have been reported to play a role in the pathogenesis of disseminated intravascular coagulation (DIC), being associated with a severe form of this disease. [15,16]

Although increased levels of plasminogen activator activity in the plasma have often been reported since Kwaan et al. [15] pointed out the case of animals infused with trypsin as a model of pancreatitis with DIC, few reports have considered the fibrinolytic activity due to plasminogen activator in the ascites during pancreatitis. In the present study, we examined the fibrinolytic system in the ascites of experimental pancreatitis and demonstrated the existence of plasminogen activator, paying particular attention to its molecular forms.

MATERIALS AND METHODS

Animals

Male Wistar rats were used for the experiments. The animals, housed at the Experimental Animal

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Center, Miyazaki Medical College, at a controlled ambient temperature of 23℃ with a relative humidity of 50%, were starved overnight but given water before undergoing treatment. Ether anesthesia was performed, as required during the experiments. Animals surviving after the completion of all procedures were kil'ed with an overdose of ether or pentobarbital sodium.

Experimental pancreatitis

Under intraperitoneal anesthesia with pentobarbital sodium (50 ㎎/㎏ body weight), acute pancreatitis was induced by retrograde infusion of 5% sodium taurocholate (Sigma Chemical Co., St. Louis, MO.) according to the method of Aho et al. [17] To assay the amidolytic activity, 10 animals weighing 380-410 g were used. Pancreatitis was induced with 0.3ml taurocholate in 5 animals (the pancreatitis group). Simple laparotomy was performed as a control in the remaining animals (the control group). In the control group, 5 ml of saline was instilled intraperitoneally immediately before fluid collection, and the collected fluid was regarded as ascites. For the other assays, 40 animals weighing 170-190 g were used, and pancreatitis was induced with 1 ml/㎏ body weight of taurocholate. Samples of ascites and blood were taken into heparinized capillaries repeatedly at known time intervals until 6 hours, or drawn into a syringe containing 1/10 volume of 3.8% trisodium citrate once at 45 minutes or 6 hours postoperatively. The samples were centrifuged at 1500 rpm for 15 minutes. The inflamed pancreas was removed after perfusion with 150 ml of saline via a catheter inserted into the aorta. The tissue was homogenized on ice with a 90-fold volume of saline for 90 seconds, followed by centrifugation at 35,000 g for 20 minutes. They supernatant was employed as the pancreas homegenate. All samples were frozen until use.

Intraperitoneal injection of pancreas homogenate

Pancreas homogenate from healthy animals was prepared as described above, except that the homogenization was with a 3-fold volume of saline and the subsequent incubation for 6 hours. It was preliminarily determined that when measured with the pancreas homegenate incubated continuously, the fibrinolvtic activity and caseinolytic activity reached a peak at 6 hours and 8 hours, respectively, and decreased thereafter. Under ether anesthesia, 3ml of pancreas homogenate, following filtration through a filter Sterivex™-GS (0.22 ㎛, Millipore Corporation, Bedford, MA.) to ensure sterility, was injected intraperitoneally into 2 healthy animals. Samples of ascites were taken at known time intervals and treated as above.

Chemicals

Synthetic p-nitroanilide (pNA) substrates consisting of pyro-Glu-Pro-Val-pNA (S-2484) for leucocytic elastase, H-D-Phe-Pip-Arg-pNA • 2HCl (S-2238) for thrombin, H-D-Val-Leu-Arg-pNA • 2HCl (S-2266) for glandular kallikrein, H-D-Val-Leu-Lys-pNA • 2HCl (S-2251) for plasmin, pyro-Glu-Gly-Arg-pNA • HCl (S-2444) for urokinase and H-D-Ile-Pro-Arg-pNA • 2HCl (S-2288) for tissue-type plasminogen activator (TPA) were purchased from KabiVitrum AB (Stockholm, Sweden), while Suc-Ala-Ala-Ala-pNA (Suc-$(Ala)_3$-pNA) for pancreatic elastase, Bz-Arg-pNA • HCl for trypsin and Bz-Tyr-pNA for chymotrypsin were from Peptide Institute, Inc. (Osaka, Japan). The protease inhibitores, soy bean trypsin inhibitor (SBTI), ovomucoid trypsin inhibitor (OTI), tosyllysine chloromethyl ketone (TLCK) tosylamide phenylethylchloromethyl ketone (TPCK), and p-chloromercuribenzoic acid (PCMB) were form Sigma; diisopropyl fluorophosphate (DEP), disodium ethylendiamine tetraacetate hydrate (EDTA) and iodoacetamide from Wako Pure Chemical Industries Ltd. (Osaka, Japan); leupeptin, chymostatin, elastatinal and pepstatin from Peptide Institute: aprotinin from Bayer (Leverkusen, West Germany); and t-4-amino-methylcyclohexane carboxylic

acid (tAMCHA) from Daiichi Seiyaku Co.,Ltd. (Tokyo,Japan).

Enzyme determinations

Amidolytic activity was determined in a 1 ml reaction system containing 0.5 mM pNA substrate employing an end-point method,based essentially on the report of Friberger, [18] and expressed in μmol of p-nitroaniline released per minute per ml of sample. When required,the activity was further expressed per the protein estimated by the method of Lowry et al. [19], using bovine serum albumin as a standard. The values were analyzed by Student's t-test.

Fibrinolytic activity was determined by the standard fibrin (plasminogen-rich fibrin) plate method of Astrup and Müllertz, [20] and when necessary, additionally by the plasminogen-poor fibrin plate method. Each 10μl of sample was dripped onto the fibrin plate,followed by incubation at 37℃ for 18 hours. The fibrinolytic activity was expressed as the lysis area (mm^2). Plasminogen-poor fibrinogen was prepared by passing plasminogenrich fibrinogen (Miles Inc.,Kankakee,IL.) through a Lys-Sepharose (Pharmacia AB,Uppsala,Sweden) column. [21]

Zymographic analysis was performed using fibrin agar plates according to the method of Granelli-Piperno and Reich. [22] Each fibrin agar plate was prepared with 8.5 ml of 1.6% fibrinogen (Sigma) treated through a Lys-Sepharose column or non-treated, 8.5 ml of 1.2% agar and 1ml of 20 U/ml thrombin. Human urine dialyzed with 0.01 M Tris-HCl,pH 7.4,and a commercial urokinase preparation were employed as a reference.

Determination of inhibition by protease inhibitors

After pre-incubation of the sample with the inhibitor solution at 37℃ for 10 minutes,the residual fibrinolytic activity was estimated by the standard fibrin plate method. The inhibitory activity was expressed as % inhibition based on a comparison of the activities with and without the inhibitor.

Affinity chromatography

Lys-Sepharose chromatography was performed according to the method of Radcliffe and Heinze. [23] A 17ml pool of ascites,which was obtained at 45 minutes after induction of pancreatitis and confirmed to possess fibrinolytic activity,was diluted with 17ml of 0.05 M Tris-HCl,pH 7.5,and applied to a column containing Lys-Sepharose (bed volume,4ml) equilibrated with the buffer. The column was washed with the buffer and the adsorbed substances were eluted with the buffer containing 0.6 M NaCl and subsequently with a linear gradient to 0.5 M arginine-HCl dissolved in the buffer containing 0.6 M NaCl. The fibrinolytic activity of each fraction (fraction volume,2ml) was determined by the standard fibrin plate method. The protein concentration was estimated from the absorbance at 280 nm. The fractions corresponding to the fibrinolytic activity peak were dialyzed with 0.15 M ammonium bicarbonate,lyophilized and reconstituted with a smaller volume of 0.05 M Tris-HCl,pH 7.5,than the initial,for the purpose of concentraion.

Trypsin treatment

Trypsin treatment was carried out by incubating 7.1 μl of the sample with 1.9 μl of trypsin (trypsin from bovine pancreas,Sigma) (0-25μg/ml) at 37℃ for 15 minutes. After the reaction was stopped by the addition of 1.5 μl of 1000 KIE/ml aprotinin,the change in fibrinolytic activity was estimated by zymograpy.

RESULTS

Amidolytic activity of the ascites and plasma in pancreatitis

Figure 1 shows the amidolytic activities of the ascites and plasma at 6 hours postoperatively. The activities of the ascites and plasma in the pancreatitis group were significantly higher than those in the control group, towards all substrates except S-2484 in the case of the ascites and S-2266 in the case of the plasma, respectively. In the pancreatitis group, the activity of the ascites was highest towards the urokinase substrate S-2444, followed by the TPA substrate S-2288. Activity towards the plasmin substrate S-2251 was also confirmed. The highest activity of the plasma was towards the thrombin substrate S-2238. Compared to the activity of the plasma towards S-2238, the activities towards S-2444 and S-2288 were lower. The comparative amidolytic activities between the ascites and plasma at 6 hours in pancreatitis are listed in Table 1. The activity of the ascites was consistently higher than that of the plasma towards each substrate. The activity ratios of the ascites to plasma towards S-2444 and S-2288 were markedly high at 43 and 52, respectively, whereas those towards other substrates, for example, the trypsin substrate Bz-Arg-pNA • HCl, chymotrypsin substrate Bz-Tyr-pNA and pancreatic elastase substrate Suc-$(Ala)_3$-pNA were found to be 11, 16 and 11, respectively.

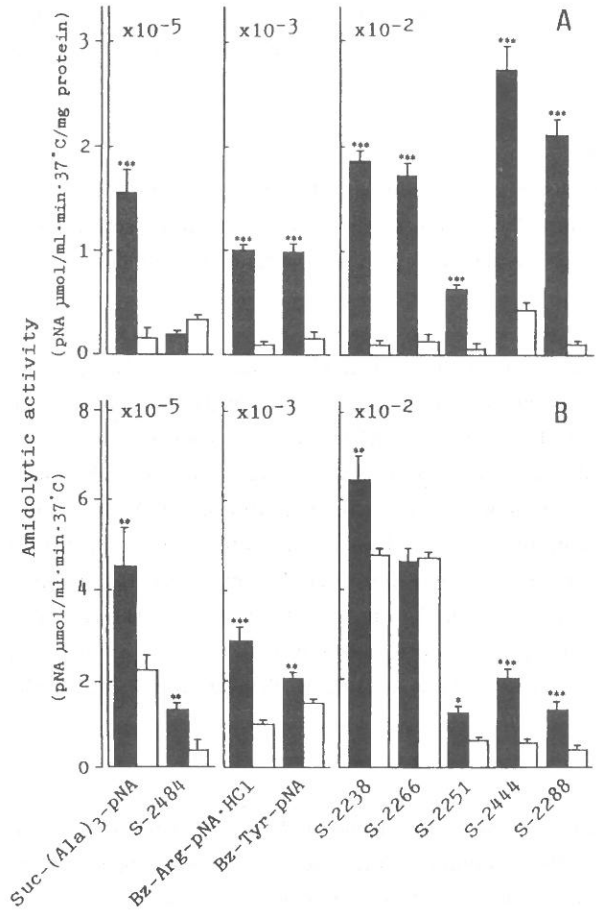


Figure 1. Amidolytic activity of ascites (A) and plasma (B) in the pancreatitis group (n=5; closed bars) and control group (n=5; open bars) at 6 hours postoperatively. The amidolytic activity of the ascites is expressed on a per protein basis. Values are presented as the mean±SE. ***$p<0.001$, **$p<0.01$, *$p<0.05$

Fibrinolytic activity of the ascties in pancreatitis

Figure 2 shows typical results for the fibrinolysis by ascites obtained at various time intervals until 6 hours. Among 18 animals with pancreatitis, fibrinolysis was observed in 8 animals (the fibrinolysis-positive group) but not in the other 10 animals (the fibrinolysis-negative group). In the fibrinolysis-positive group, the fibrinolytic activity decreased with the time course after reaching a maximum at 30 minutes or 1 hour; that is, it disappeared at 2 hours in 2, at 4 hours in 2, and at 6 hours in 3 animals. In 1 animal, the activity continued until 6 hours, as shown in Figure 2. A lower activity was also detected on the plasminogen-poor fibrin plate. No fibrinolysis was observed in plasma obtained before or after the induction of pancreatitis.

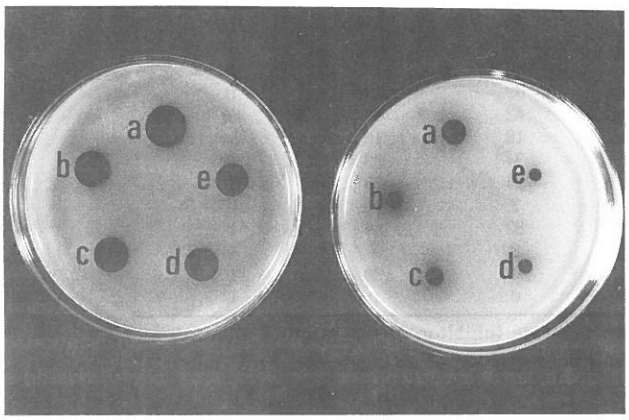


Figure 2. Fibrinolysis by ascites of pancreatitis on a plasminogen-rich fibrin plate (left) and a plasminogen-poor fibr in plate (right). The ascites was obtained at 30 minutes (a), 1(b), 2(c), 4(d) and 6 hours (e) post-operatively.

Table 1. Comparison of amidolytic activity between the ascites and plasma in pancreatitis

Substrate	Amidolytic activity (pNA μmol/ml·min·37℃)		Ascites/plasma ratio
	Ascites	Plasma	
Suc-$(Ala)_3$-pNA	4.9×10^{-4}	4.5×10^{-5}	11
S-2484	5.9×10^{-5}	1.2×10^{-5}	5
Bz-Arg-pNA·HCl	3.2×10^{-3}	2.9×10^{-4}	11
Bz-Tyr-pNA	3.1×10^{-3}	2.0×10^{-4}	16
S-2238	5.9×10^{-1}	6.4×10^{-2}	9
S-2266	5.6×10^{-1}	4.6×10^{-2}	12
S-2251	2.0×10^{-1}	1.3×10^{-2}	15
S-2444	8.6×10^{-1}	2.0×10^{-2}	43
S-2288	6.7×10^{-1}	1.3×10^{-2}	52

Values are presented as the mean. The ascites and plasma were obtained at 6 hours postoperatively.

Comparative effects of protease inhibitors

The inhibitory effects of various protease inhibitors were estimated using 2-fold diluted ascites with fibrinolytic activity and non-diluted pancreas homogenate, both obtained at 45 minutes after the induction of pancreatitis. A commercial urokinase preparation was employed as the reference. The result

are summarized in Table 2. The inhibitory effects on the ascites and pancreas homogenate were similar to each other with several exceptions. Although the activity of urokinase was not inhibited by chymostatin, OTI, TLCK, TPCK and EDTA, the activity of the ascites was inhibited by them. All the activities of the ascites, pancreas homogenate and urokinase were strongly inhibited by SBTI and DFP.

Table 2. Comparative effects of protease inhibitors against fibrinolytic activity

Protease inhibitor		% inhibition		
		Ascites	Pancreas homogenate	Urokinase
SBTI	(1 mg/ml)	100	100	100
DFP	(10 mM)	83	100	100
Leupeptin	(0.1mM)	100	100	36
Chymostatin	(0.1mM)	60	78	0
OTI	(1 mg/ml)	69	100	0
Aprotinin	(100 KIE/ml)	42	47	69
tAMCHA	(10 mM)	18	16	54
TLCK	(1 mM)	34	33	0
TPCK	(1 mM)	24	79	1
Elastatinal	(10 mM)	7	75	17
EDTA	(1 mM)	32	49	0
Iodoacetamide	(10 mM)	8	7	0
PCMB	(1 mM)	0	20	0
Pepstatin	(0.1 mM)	0	0	0

The final concentration of each protease inhibitor in the reaction mixture is indicated in parentheses. The final concentration of urokinase was 10 IU/ml.

Zymography of the ascites during pancreatitis

Figure 3 illustrates the zymography of the ascites obtained from 1 animal in the fibrinolysis-positive group as an example. Fresh dialyzed urine revealed lytic zones at about 100,000 and 50,000 molecular weight positions. Commercial urokinase preparatin showed the lytic zone at about the 50,000 and 30,000 molecula weight positions. When samples of the ascites from 6 animals in the fibrinolysis-positive group were analyzed by zymography using plasminogen-rich fibrin agar plates, the major lytic zone was observed in the 50,000 molecular weight region from 30 minutes until 6 hours after the induction of pancreatitis in all the animal (Figure 3). The molecular weight of the lytic zone gradually increased with the time course. A similar phenomenon was also observed for samples of the ascites from 3

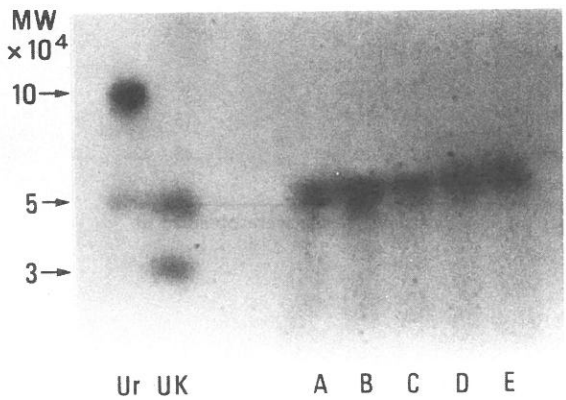


Figure 3. Zymography of ascites in pancreatitis. Ur, human urine. UK, commercial urokinase preparation. (A)-(E), ascites (obtained at 30 minutes (A), 1 (B), 2 (C), 4 (D), and 6 hours (E) postoperatively). The volume of ascites in each slot was 5.0μl.

animals in the fibrinolysis-negative group (date not shown). In other experiments, no lytic zone at this position was detected on zymography using plasminogen-poor fibrin agar plates (date not shown).

Affinity chromatography and effect of trypsin

The profile on Lys-Sepharose chromatography of the ascites of pancreatitis is illustrated in Figure 4. Peaks of fibrinolytic activity were observed in each of the non-adsorption fractions, NaCl elution fractions and arginie elution fractions. These peak fractions were concertrated 10-fold, 10-fold and 30-fold, respectively. Zymographic patterns of the fractions before and after concentration are shown in Figure 5. Before concentration, the non-adsorption fraction revealed a broad lytic zone in the 50,000-80,000 molecular weight range and the NaCl elution fraction revealed lytic zones at the 100,000 molecular weight position, in the 50,000-100,000 molecular weight range and at a position much higher than molecular weight 100,000. After concentration, these two fractions demonstrated a lytic zone at the same position of molecular weight 50,000. The arginine elution fraction did not exhibit any activity on zymography before or after concentration. The fibrinolytic activity of the NaCl elution fraction after concentration was increased by trypsin treatment, as illustrated in Figure 6. No activity was observed on zymography using plasminogen-poor fibrin agar plates with the non-adsorption fraction or the NaCl elution fraction after concentration (data not shown).

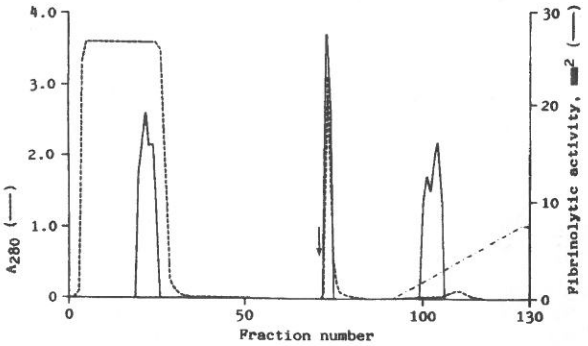


Figure 4. Affinity chromatography of ascites of pancreatitis on Lys-Sepharose. Ascites diluted with 0.05 M Tris-HCl, pH 7.5, was applied. After the column was washed with the buffer, elution was performed with the buffer containing 0.6 M NaCl(↓)and with the addition of a linear gradient to 0.5 M arginine-HCl dissolved in the buffer containing 0.6 M NaCl (— • — • —). The fibrinolytic activity of each fraction was determined by the standard fibrin plate method. The protein concentration was measured from the absorbance at 280 nm.

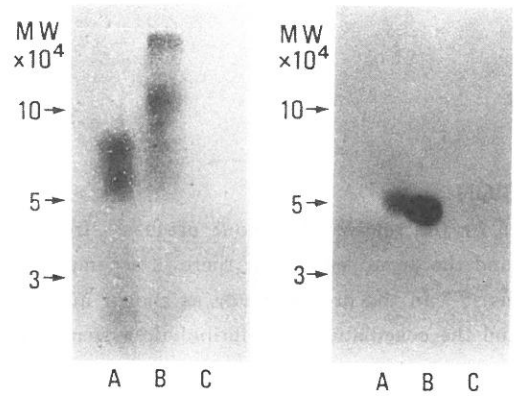


Figure 5. Molecular forms of fibrinolytic enzymes in the non-adsorption fraction (A), NaCl elution fraction (B) and arginine elution fraction (C) separated from the ascites by Lys-Sepharose chromatography. The patterns before concentration are shown on the left and those after concentration on the right.

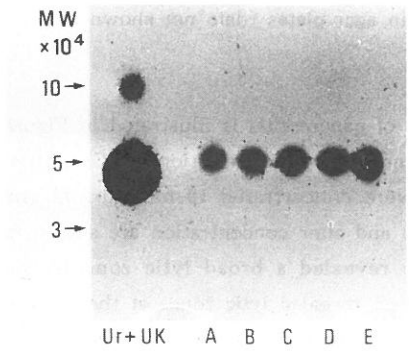
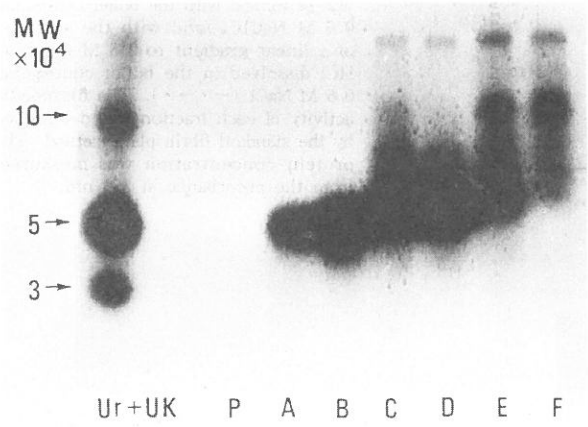


Figure 6. Effect of trypsin on the NaCl elution fraction after concentration. 7.1 μl of the fraction was incubated with 1.9 μl of trypsin at 37℃ for 15 minutes. After the reaction was stopped by the addition of 1.5 μl of 1000 KIE/mℓ aprotinin, the mixture was subjected to zymography. Concentration of added trypsin: (A) 0 μg/mℓ, (B) 3.1 μg/mℓ, (C) 6.3 μg/mℓ, (D) 12.5 μg/mℓ, and (E) 25 μg/mℓ.

Zymography of the ascites following injection of pancreas homogenate

When the pancreas homogenate was injected intraperitoneally into healthy rats, the ascites exhibited a lytic zone in the 50,000 molecular weight region from 10 minutes until 6 hours after the injection on zymography (Figure 7). The lytic zone appeared to shift to a higher molecular weight position with the time course. In addition, the ascites showed lytic zones at the 100,000 molecular weight position at 1, 4 and 6 hours and lytic zones at a position much higher than molecular weight 100,000 from 1 until 6 hours after the injection. Several lytic zones within the 50,000 to 100,000 molecular weight range were also observed. The pancreas homogenate itself did not display any activity on zymography.



Figuer 7. Zymography of the ascites following injection of pancreas homogenate. Ur, dialyzed human urine. UK, commercial urokinase preparation. (P) pancreas homogenate, (A) - (F) ascites (obtained at 10 (A), 30 minutes (B), 1 (C), 2 (D), 4 (E) and 6 hours (F) after injection). The volume of ascites in each slot was 5.0 μl.

DISCUSSION

During acute pancreatitis, which is characterized by the release of various proteases from the autocatalyzed pancreas into the systemic circulation and the peritoneal cavity, there is accompanying abnormality of the coagulation and fibrinolytic systems.[24-26] In the present study, as shown in Figure 1, increased activities of proteases on the pancreas and the coagulation and fibrinolytic systems were confirmed in the plasma of pancreatitis induced in rats using various synthetic substrates. Similar phenomena were observed in the ascites of pancreatitis. In particular, the activities of trypsin, chymotrypsin, plasmin and plasminogen activators which were related to fibrinolysis were increased using synthetic substrates, and the fibrinolytic activity observed in the ascites (Figure 2) apparently depended on the high activities of these proteases. The amidolytic activities of the ascites were markedly higher than those of the

plasma (Table 1). Dubick et al. [9] reported the existence of an immunological imbalance between pancreatic proteases and protease inhibitors in ascites obtained from patients with acute pancreatitis. The high activity of the ascites in the preaent study may reflect such an imbalance. The activity ratios of the ascites to plasma towards urokinase and TPA substrates were markedly high, as compared with the ratios towards other subdtrates. This clearly suggested that urokinase and TPA were increaded in the ascites, as compared with the plasma.

When subjected directly to zymography, the ascites revealed a lytic zone in the 50,000 molecular weigh region (Figure 3). This zone appeared to be due to plasminogen activator, since no corresponding lytic zone was observed on zymography using plasminogen-poor fibrin agar plates. The lytic zone shifted to a higher molecular weight position with the time course. This phenomenon appeared to indicate that the lytic band at a higher molecular weight position was equivalent to a form of plasminogen activator which was more closely allied to the 100,000 molecular weight plasminogen activator which was demonstrated in the NaCl fraction by zymography.

Of the three fractions of the ascites, the non-adsorption fraction, NaCl elution fraction and arginine elution fraction, separated by Lys-Sepharose affinity chromatography, each of the former two fractions exhibited a lytic zone at the 50,000 molecular weight position on zymography after concentration (Figure 5). The absence of a corresponding lytic zone on zymography when using plasminogen-poor agar plates would seem to indicate that this lytic zone was due to plasminogen activator. Also, the 50,000 molecular weight of the plasminogen activator was almost in accord with the molecular weight of rat urine urokinase as reported previously. [27] Concerning the affinity for lysine, it has been found that urokinase did not bind to lysine, whereas most pro-urokinase did bind to lysine. [28] Similarly, in the case of fibrin-binding, urokinase had little or no affinity and pro-urokinase had a strong affinity. [28, 29] In the present study, the peak of fibriolytic activity in the non-adsorption fractions appeared at the late stage of these fractions on Lys-Sepharose chromatography (Figure 4). This finding may indicate that the plasminogen activator in the non-adsorption fraction had a very weak affinity for lysine. The plasminogen activator in the NaCl elution fraction had a clear affinity for lysine, and its activity was increased by trypsin treatment on zymography after concentration (Figure 6). This phenomenon suggests that the NaCl elution fraction after concentration may have contained the proenzyme form of plasminogen activator. The effect of trypsin on the proenzyme form of urokinase was reported previously to involve its activation. [30,31] By zymography, the NaCl elution fraction before concentration contained a 100,000 molecular weight plasminogen activator (Figure 5). Concerning such large molecular weight plasminogen activators, Vetterlein et al. [32] demonstrated the existence of a 90,000 molecular weight plasminogen activator. Stump et al. [33] reported a 95,000 molecular weight urokinase-related protein lacking in fibrin-binding as being a 54,000 molecular weight urokinase-inhibitor complex. On the other hand, Harvey et al. [34] proposed that urokinase-like enzymes with unusually large molecular weights, which were broken down to smaller active fragments by trypsin, might represent zymogen forms. Sumi et al. [35] described a fibrin-binding urokinase (precursor form) with a molecular weight of 100,000 in human urine, the activity of which was enhanced by trypsin and plasmin. The appearance of multiple molecular forms in the present study might be interpreted as indicating that the 100,000 molecular weight plasminogen activator is a zymogen of urokinase, this zymogen was degraded to active substances with molecular weights in the range of 50,000-80,000 and 50,000-100,000 in the peritoneal cavity, and furthermore the substances were converted to pro-urokinase or urokinase during the procedure of concentration.

Detailed information on the fibrinolytic enzymes in the arginine elution fraction was not obtained except regarding their very strong affinity for lysine. Such a strong affinity is reminiscent of the affinity

of TPA for lysine [23] and fibrin [36, 37] as reported previously. The plasminogen activators, urokinase and TPA, can be distinguished from each other immunologically. An immunological method is available for the identification of the various types of human plasminogen activators. [38] Since the antibodies for rat plasminogen activators could not be obtained, a complete determination of the types by the immunological method could not be achieved in this study.

The increase of plasminogen activator activity in the ascites of pancreatitis was thought to be induced by the substances released from the pancreas into the peritoneal cavity during pancreatitis. To test this supposition, experiments involving the injection of pancreas homogenate were carried out. The zymographic pattern of the ascites following the injection of pancreas homegenate consisted of 3 lytic zones in the 50,000 molecular weight region, at the 100,000 molecular weight position and at a position much higher than molecular weight 100,000 as well as several lytic zones within the 50,000 to 100,000 molecular weight range (Figure 7), as observed for the ascites of pancreatitis. Moreover, the lytic zone observed in the 50,000 molecular weight region appeared to shift to a higher molecular weight position with the time course, similarly to the ascites of pancreatitis. Such similarities suggest that the substances released from the pancreas promoted an increase of plasminogen activator in the ascites of pancreatitis. It is well known that proteases can release plasminogen activator in the endothelium. [39] Pancreatic proteases such as trypsin and chymotrypsin probably released plasminogen activators from the peritoneum, at least partly, in the zymogen form. The appearance of the multiple molecular forms of plasminogen activator which were observed in the ascites of pancreatitis, might be brought about by the action of pancreatic proteases.

The local role of plasminogen activator in ascites is unknown. Although it is possible that the plasminogen activator as well as other fibrinolytic enzymes participate in a change of the fibrinolytic system in the blood by transfer into the systemic circulation, the extent of such participation remains to be determined.

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