

A Factor X-Activating Cysteine Protease from Malignant Tissue

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ABSTRACT A proteolytic procoagulant has been identified in extracts of human and animal tumors and in cultured malignant cells. It directly activated Factor X but its similarity to other Factor X-activating serine proteases was not clear. This study describes work done to determine whether this enzyme, cancer procoagulant, is a serine or cysteine protease. Purified cancer procoagulant from rabbit V2 carcinoma was bound to a *p*-chloromercurialbenzoate-agarose affinity column and was eluted with dithiothreitol. The initiation of recalcified, citrated plasma coagulation activity by cancer procoagulant was inhibited by 5 mM diisopropylfluorophosphate, 1 mM phenylmethylsulfonylfluoride, 0.1 mM HgCl₂, and 1 mM iodoacetamide. Activity was restored in the diisopropylfluorophosphate-, phenylmethylsulfonylfluoride-, and HgCl₂-inhibited samples by 5 mM dithiothreitol; iodoacetamide inhibition was irreversible. Russell's viper venom, a control Factor X-activating serine protease, was not inhibited by either 0.1 mM HgCl₂ or 1 mM iodoacetamide. The direct activation of Factor X by cancer procoagulant in a two-stage assay was inhibited by diisopropylfluorophosphate and iodoacetamide. Diisopropylfluorophosphate inhibits serine proteases, and an undefined impurity in most commercial preparations inhibits cysteine proteases. Hydrolysis of diisopropylfluorophosphate with CuSO₄ and imidazole virtually eliminated inhibition of thrombin, but cancer procoagulant inhibition remained complete, suggesting that cancer procoagulant was inhibited by the undefined impurity. These results suggest that cancer procoagulant is a cysteine endopeptidase, which distinguishes it from other coagulation factors including tissue factor. This and other data suggest that neoplastic cells produce this unique cysteine protease which may initiate blood coagulation.

INTRODUCTION

Factor X is the point of confluence of the intrinsic and extrinsic pathways in the coagulation cascade. Factor X

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is converted to its activated form (Factor Xa) by the proteolytic removal of an activation glycopeptide with a molecular weight of ~11,000 (1, 2). This activation can be facilitated by either Factors IXa and VIII, Factor VIIa and tissue factor, the autolytic conversion by Factor Xa, or by an enzyme in the venom of *Vipera Russellii* (Russell's viper venom) (1-4). The active component within all of these Factor X-activating systems is a serine protease (1-5). In addition, Factor X can be activated by other serine proteases such as trypsin (1, 2, 6), cathepsin C (7), and the plant cysteine protease, papain (8).

Increased vascular thrombosis and the deposition of fibrin associated with malignant disease led us to look for an abnormal coagulation factor associated with neoplastic tissue. A proteolytic enzyme, cancer procoagulant, has been purified from rabbit V2 carcinoma and some of its physical, chemical, and enzymatic properties have been determined.¹ In addition, cancer procoagulant activity has been identified in extracts of several human malignant tumors (9) and in cultured malignant cells² (10-13): This enzyme is inhibited by diisopropylfluorophosphate (DFP)³ and initiates coagulation in the absence of Factor VII by directly activating Factor X (14). Thus, we assumed that cancer procoagulant was a serine protease, similar to the other enzymes that activate Factor X. These studies provide data which indicate that cancer procoagulant is not a serine protease but is a cysteine protease.

METHODS

Materials. A 0.5-M stock solution of DFP (Aldrich, Chemical Co., Milwaukee, Wis.) was prepared in dry iso-

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³ *Abbreviations used in this paper:* DFP, diisopropylfluorophosphate; DTT, dithiothreitol; MES, 2-[*N*-morpholine]ethane sulfonic acid; PCMB, *p*-chloromercurialbenzoate-agarose; PMSF, phenylmethylsulfonylfluoride.

propanol and diluted 1:100 in the sample to facilitate inhibition. A stock solution of 0.01 M HgCl₂ was prepared in water and added to samples in a 1:100 ratio to a final concentration of 0.1 mM. A stock solution of 0.1 M phenylmethylsulfonylfluoride (PMSF) (Sigma Chemical Co., St. Louis, Mo.) was prepared in dry dioxane and added to samples in a 1:100 ratio to a final concentration of 1 mM. A 1 × 20-cm *p*-chloromercurialbenzoate-agarose (PCMB) (Bio-Rad Laboratories, Richmond, Calif.) affinity column was prepared in 20 mM 2[*N*-morpholino]ethane sulfonic acid (MES) buffer (pH 6.8) to test the efficiency of cancer procoagulant binding and for the fractionation of the protease.

Citrated bovine and human plasma were obtained fresh and prepared as described previously (14) for use in the single-stage recalcified coagulation time assay. Pure bovine Factor X was kindly provided by Dr. Earl Davie and used in the two-stage clotting assay as described previously (14). Russell's viper venom (Sigma Chemical Co.) was used as a control Factor X-activating serine protease. Partially purified bovine thrombin (Parke Davis and Co., Detroit, Mich.) was diluted to 0.1 U/ml in 10 mM veronal-buffered saline (pH 7.6).

Methods. General: Cancer procoagulant was purified from rabbit V2 carcinoma tumor tissue.⁴ Briefly, tumor tissue was removed surgically before the animal's death, extracted in three changes of 20 mM veronal buffer (pH 7.8) for 3 h each, and the pooled extracts were concentrated about 10-fold over a PM-10 ultrafiltration membrane. The concentrated extract was applied to a benzamidine affinity chromatography column, unbound protein was washed from the column with 10 mM veronal buffer (pH 7.9) containing 1 mM EDTA and 50 mM NaCl, and the bound protein was eluted with 1.0 M propionic acid. After neutralizing the propionic acid with NaOH, the sample was concentrated on a PM-10 ultrafiltration membrane, dialyzed, and applied to a 1.5-agarose gel filtration column. The high molecular weight, included protein peak containing the procoagulant activity was reappplied to a benzamidine affinity resin, the unbound protein was washed from the column with 10 mM veronal buffer, adsorbed protein was eluted with 0.1% Triton X-100, and then the bound protein was eluted with 0.5 M propionic acid. After neutralizing and removing the propionic acid by dialysis, the concentrated sample was applied to a phenyl-Sepharose hydrophobic affinity column and the purified procoagulant was eluted with 10% dimethyl sulfoxide. The purified procoagulant sample was homogeneous by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. At each step of the purification the procoagulant activity was assayed by measuring the recalcified coagulation time of citrated plasma (14) and its protein concentration with a modified Lowry method (15). Its sensitivity to DFP and its activity in Factor VII-depleted bovine plasma (14, 16) were determined.

Affinity chromatography on PCMB resin. PCMB affinity resin was equilibrated in 25 mM MES buffer (pH 6.8), packed in a 1 × 10-cm column and run at a flow rate of ~1 ml/min. About 1.0 ml of a sample eluted from the second benzamidine affinity column was applied to the PCMB affinity resin in 20 mM veronal buffer (pH 7.7) and washed onto the column with 40 ml of MES buffer (pH 6.8), 40 ml of 1 M urea and 1% Tween 20 in MES buffer, 20 ml MES buffer to clear the urea and Tween from the column, 35 ml of 0.1 mM dithiothreitol (DTT), 35 ml of 5 mM DTT, 35 ml of 10 mM DTT, and finally 35 ml of 100 mM DTT to strip the column of residual protein. Protein eluted from the column was continuously monitored at 280 nm with an ISCO model UA-5 absorption monitor (Instrumentation Specialties Co., Lincoln, Neb.).

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The protein peaks were collected separately and concentrated ~10-fold on a PM-10 ultrafiltration membrane and dialyzed against MES buffer to remove the DTT. These samples were assayed for protein and procoagulant activity as described above.

Inhibition studies. Samples purified by the method described above and those obtained from the PCMB affinity column were tested for their inhibition by 5 mM DFP, 1 mM PMSF, 1 mM iodoacetamide, and 0.1 mM HgCl₂. In each case, samples of cancer procoagulant were assayed for procoagulant activity by measuring the single-stage recalcification clotting time of citrated human plasma with a fibrometer. The percent decrease in the clotting time of samples containing cancer procoagulant or controls compared to the appropriate 25 mM MES buffer blank was used to quantify coagulant activity. Russell's viper venom was used as a coagulation control and as a Factor X-activating serine protease control. Portions of the samples and controls were treated with the appropriate inhibitors, incubated for 30 min at 37°C, and reassayed for procoagulant activity. Portions of each sample were made 5 mM with DTT, incubated overnight at 5°C, dialyzed for 3 h against two changes of 25 mM MES buffer, and reassayed for procoagulant activity to determine if the inhibition had been effectively reversed by the reducing agent. In all studies, the activity of the untreated samples was compared with the same sample after inhibition and reactivation by a paired *t*-test analysis.

To determine whether cancer procoagulant was inhibited by DFP or by the conjectured impurity of DFP, samples of DFP were treated with cupric ion and imidazole to catalyze the hydrolysis of DFP (17) and tested for their inhibitory capacity at various times during the hydrolysis reaction (18). A solution of 25 mM CuSO₄ and 25 mM imidazole was prepared in water, adjusted to pH 7.6 with 1 N NaOH, and made 10 mM DFP by the addition of 0.5 M DFP in isopropanol. The reaction mixture was incubated at 37°C for 2 h with constant stirring. Samples (0.2 ml) of the reaction mixture were taken at 0, 60, and 120 min, pH was adjusted to 7.6, and 20 μl of 250 mM EDTA was added to stop the hydrolysis of DFP. A sample (0.1 ml) of thrombin or cancer procoagulant was mixed with an equal volume of the EDTA-treated reaction mixture, incubated for 30 min at 37°C, and assayed by measuring the recalcified clotting time as described above. Control thrombin, cancer procoagulant and buffer blank samples were mixed with the same amounts of CuSO₄, imidazole, and EDTA, but containing isopropanol without DFP to determine the effect of these ingredients on the clotting assay.

The direct activation of Factor X. A two-stage coagulation assay was used, as described previously (14), to confirm that the protein eluted from the PCMB column and sensitive to the inhibitors could directly activate Factor X. In brief, purified bovine Factor X was incubated with either cancer procoagulant or Russell's viper venom in the first stage of the clotting assay. Samples were removed from the first stage and added to Factor VII- and Factor X-deficient bovine plasma (Sigma Chemical Co.) and the recalcified clotting time of the second stage was measured with a fibrometer. Both inhibited and uninhibited samples were assayed for their ability directly to activate Factor X. The results are expressed as the normalized clotting time of the second stage of the assay.

RESULTS

PCMB-affinity chromatography. A partially purified V2 carcinoma extract sample containing cancer procoagulant was applied to the PCMB affinity column (Fig. 1). Protein was eluted in each of the six washes.

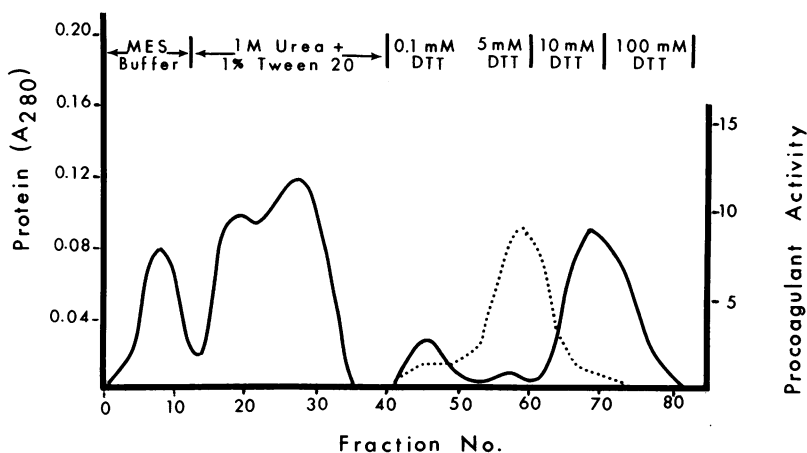


FIGURE 1 An elution profile of the PCMB chromatography column. A partially purified rabbit V2 carcinoma extract was applied to the affinity column. The column was eluted with 40 ml of 25 mM MES Buffer (pH 6.8), 40 ml of 1 M urea and 1% Tween 20 in 25 mM MES buffer; urea and Tween 20 were washed off the column with 20 ml of MES buffer; and the bound protein was eluted with 35 ml of 0.1 mM DTT, 35 ml of 5 mM DTT, 35 ml of 10 mM DTT, and 35 ml of 100 mM DTT. Eluted protein (—) was monitored by reading absorption at 280 nm and procoagulant activity (·····) was assayed by measuring the recalcification clotting time of normal citrated human plasma.

A small amount of procoagulant activity was eluted in 0.1 mM DTT with the major peak of activity in the 5 mM eluate.

Inhibition studies. Studies of the inhibition characteristics of purified cancer procoagulant were performed in an effort to determine whether it was a serine or cysteine protease. Purified cancer procoagulant was treated with 5 mM DFP, 1 mM PMSF, 0.1 mM HgCl_2 , and 1 mM iodoacetamide. The inhibited samples were assayed for procoagulant activity (Table I). Treatment of the inhibited enzyme with 5 mM DTT effectively reactivated samples inhibited with DFP, PMSF, and HgCl_2 . Iodoacetamide inhibition was irreversible. Russell's viper venom, the control Factor X-activating serine protease, was treated with 0.1 mM HgCl_2 and 1 mM iodoacetamide. It was not inhibited by either HgCl_2 or iodoacetamide.

To determine whether iodoacetamide treatment might denature cancer procoagulant rather than block its active site, a PMSF-treated sample was made 1 mM with iodoacetamide and then treated with 5 mM DTT, dialyzed, and analyzed for activity. There was no procoagulant activity after treatment with PMSF or after iodoacetamide treatment, but full activity was recovered after reductive DTT reactivation. A similar double inhibition was performed to determine whether DFP and iodoacetamide reacted at the same site on the enzyme. A DFP-inhibited sample was made 1 mM with iodoacetamide and then reactivated with 5 mM DTT. There was no clotting activity after DFP and iodoacetamide treatment but reactivation with DTT facilitated recovery of full activity, whereas the inhibition

of the control cancer procoagulant sample, treated with iodoacetamide alone, was irreversible.

Cupric ion and imidazole catalyze the hydrolysis of DFP with the release of hydrofluoric acid (17); in these experiments the decrease in pH confirmed that the reaction was proceeding. In four experiments, the clotting time of the untreated thrombin control and the purified cancer procoagulant sample was 112 ± 4 and 130 ± 8 s, respectively, after normalizing the results to a 200-s blank time. Thrombin and cancer procoagulant were treated with the DFP hydrolysis mixture immediately after it was prepared (0 min) and after 60 and 120 min incubations at 37°C . Thrombin activity decreased to $9 \pm 6\%$ of its original clotting activity after reacting with the 0-min DFP hydrolyzate (Fig. 2). After treatment with the 60- and 120-min DFP hydrolyzate, thrombin activity was about 50 and 92% of its original level, respectively. In contrast, cancer procoagulant activity was almost completely inhibited after treatment with 0-, 60-, and 120-min DFP hydrolyzate. These results suggest that the inhibitor of thrombin was hydrolyzed by the CuSO_4 -imidazole reaction, whereas the cancer procoagulant inhibitor was not destroyed under these conditions.

To confirm that the procoagulant activity blocked by the inhibitors was activating Factor X as previously described (10), a two-stage clotting assay was performed. Both DFP and iodoacetamide effectively inhibited the activation of Factor X by cancer procoagulant in the first stage of the assay (Fig. 3). Iodoacetamide did not affect the activity of the Russell's viper venom control as expected.

TABLE I
The Effect of Various Inhibitors on the Coagulation Activity of Russell's Viper Venom and Purified Cancer Procoagulant

	n	Clotting time		Mean difference	P	Inhibited %
		Untreated	Treated			
Cancer procoagulant						
DFP						
inhibited	7	175±3	201±4	26±1	<0.005	100
reactivated	3	181±2	168±6	-13±6	NS	0
PMSF						
inhibited	6	175±4	207±10	32±11	<0.025	100
reactivated	5	171±3	167±7	-4±8	NS	0
HgCl ₂						
inhibited	6	168±5	204±5	36±2	<0.005	100
reactivated	5	172±4	176±7	4±4	NS	14
IA						
inhibited	8	172±4	200±2	28±5	<0.005	100
reactivated	3	166±9	202±8	36±4	<0.005	100
Russell's viper venom						
HgCl ₂						
inhibited	3	159±11	160±5	1±14	NS	0
IA						
inhibited	5	157±6	146±15	-11±18	NS	0

Samples were made 5 mM DFP, 1 mM PMSF, 0.1 mM mercuric chloride (HgCl₂), or 1 mM iodoacetamide (IA) and incubated for 30 min at 37°C. To reactivate the inhibited coagulant activity, portions of the samples were made 5 mM DTT, incubated at 5°C overnight, and dialyzed for 3 h against 25 mM MES buffer (pH 6.8). The untreated samples, inhibitor-treated samples, and reactivated samples were assayed for activity by measuring the recalcified clotting time of normal citrated human plasma. The results are expressed as the mean clotting time of (n) untreated and treated samples±SEM normalized to a blank clotting time of 200 s. The mean difference±SE of the difference between the activities of the untreated sample and its inhibited or reactivated counterpart was compared to a paired *t* test. The relative change in activity after inhibition and reactivation is expressed as a percent inhibition.

DISCUSSION

Recently, cancer procoagulant was purified and some of its physicochemical characteristics were determined (14). It is a proteolytic enzyme that is inhibited by DFP, facilitates clotting of Factor VII-depleted plasma, and initiates coagulation by directly activating Factor X in the coagulation cascade. Until recently, it was assumed that cancer procoagulant was a serine protease because it was inhibited by 5 mM DFP, there was effective binding of cancer procoagulant to a benzamidine-Sepharose affinity column, and it mimicked the behavior of other coagulation serine proteases (Factors IXa and VIIa and the Factor X-activating protease in Russell's viper venom) that are known to activate Factor X (1-5). However, during studies to determine the optimum conditions for the release of tritium-labeled activation peptide from ³H-Factor X (19) by cancer procoagulant, the optimum pH for the

activation of Factor X was found to be ~6.5.⁵ Since serine proteases are more active between pH 7 and 9 (20), and cysteine proteases (thiol and sulfhydryl proteases) are more active between pH 5 and 7 (20, 21), this observation suggested that cancer procoagulant might be a cysteine protease. This led us to study the effect of specific inhibitors on cancer procoagulant activity in an effort to determine whether cancer procoagulant was a serine or cysteine protease.

Cysteine proteases are inhibited by PCMB and will bind to a PCMB ligand linked to agarose (22, 23). The reasonably specific nature of the binding of cysteine proteases to a PCMB-agarose affinity column provides evidence suggesting that cancer procoagulant may be a cysteine protease. Free sulfhydryl groups on other proteins will bind to a PCMB affinity resin (24, 25), but the specificity and avidity of this binding may be less

⁵ Manuscript in preparation.

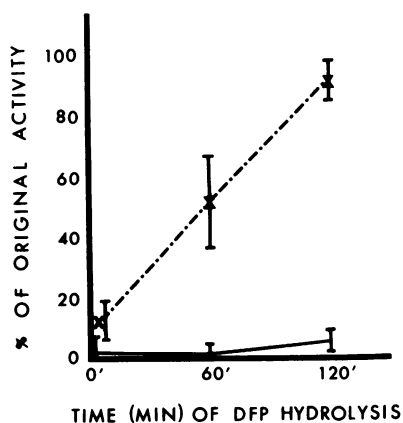


FIGURE 2 Effect of DFP hydrolysis on the inhibition of thrombin and cancer procoagulant. Equimolar amounts of CuSO_4 and imidazole (25 mM) were incubated with 10 mM DFP at 37°C for 2 h to catalyze the hydrolysis of DFP to diisopropylphosphate and HF. Samples (0.2 ml) of the hydrolyzate were removed immediately after preparing the reaction mixture (0 min) and after 60 and 120 min, and the reaction was stopped with $20\ \mu\text{l}$ of 250 mM EDTA. Thrombin ($\cdots\cdots$) or cancer procoagulant (—) samples (0.1 ml) were mixed with 0.1 ml of the hydrolyzate, incubated for 30 min at 37°C , and assayed by measuring the clotting time of recalcified citrated plasma. The results are expressed as the mean percent of activity in the initial, untreated thrombin or cancer procoagulant sample \pm SEM ($n = 4$).

than when the active site of an enzyme recognizes the PCMB ligand. The results demonstrate that cancer procoagulant binds to the PCMB affinity resin and is not eluted by 1 M urea and 1% Tween 20 with the other adsorbed proteins and is effectly removed with DTT, properties that are consistent with the concept that cancer procoagulant may be a cysteine protease.

One of the effective ways to distinguish between serine and cysteine proteases is to inactivate them selectively by means of specific inhibitors (20, 21). DFP and PMSF are well-known irreversible inhibitors of serine proteases (20, 26, 27); PMSF is a less effective inhibitor of cysteine proteases, and is reversible (20, 21, 28); and the DFP inhibition of cysteine proteases is controversial, as discussed below. Iodoacetamide and mercury are more specific inhibitors for cysteine proteases and are less effective or even ineffective for the inhibition of serine proteases (20–22). Cancer procoagulant was inhibited by all four inhibitors, and the inhibition by DFP, PMSF, and mercury was reversed by reductive removal of the inhibitors. Inhibition by iodoacetamide was irreversible. To determine whether the inhibition by iodoacetamide might be altering the protein by means other than of active site inhibition (e.g., by facilitating a conformational change by reacting with nonactive site sulfhydryl groups), cancer procoagulant was first inhibited by PMSF to block the active site, and then the inhibited cancer procoagulant was reacted with iodoacetamide. After reductive

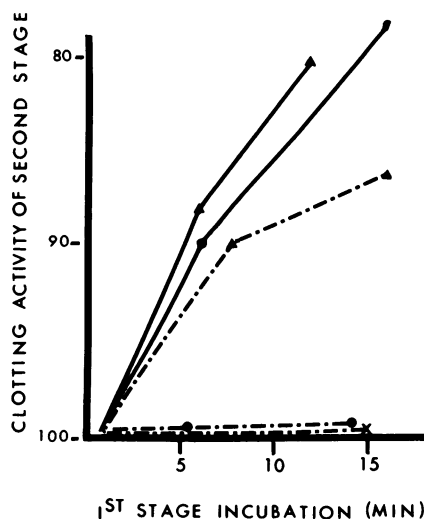


FIGURE 3 The direct activation of Factor X by cancer procoagulant and Russell's viper venom in a two-stage coagulation assay. The first stage of the assay contains $0.2\ \mu\text{g}$ of purified bovine Factor X in 0.5 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 5 mM CaCl_2 and 0.15 M NaCl, $3\ \mu\text{g}$ crude rabbit brain cephalin in $20\ \mu\text{l}$ saline and either 5 ng of purified cancer procoagulant (\triangle - - - \triangle) or 1 pg of Russell's viper venom control (\triangle — \triangle). To determine the amount of Factor Xa generated, timed samples ($100\ \mu\text{l}$) of the first stage reaction mixture were mixed with $100\ \mu\text{l}$ of Factor VII- and Factor X-depleted bovine plasma and $100\ \mu\text{l}$ of 20 mM CaCl_2 and the clotting time of the second stage was measured with a fibrometer. To determine the effect of the inhibitor on the direct activation of Factor X, samples of the Russell's viper venom and cancer procoagulant were treated with 1 mM iodoacetamide (\bullet). Cancer procoagulant was adjusted to contain 5 mM DFP (\times), the treated samples were incubated for 30 min at 37°C and assayed in the two-stage clotting assay. The clotting activity of the second stage is expressed as the normalized clotting time (seconds) of the Russell's viper venom and cancer procoagulant samples.

removal of the PMSF from the active site of cancer procoagulant, all its activity was recovered, suggesting the iodoacetamide was not causing inactivation by reacting with other sites on the proteolytic enzyme. A similar study in which DFP inhibition effectively blocked the ability of iodoacetamide to inhibit cancer procoagulant activity irreversibly suggests that DFP and iodoacetamide are reacting at the same site, or at closely adjacent sites in the enzyme.

Several investigators have examined the DFP inhibition of plant cysteine proteases, such as bromelain (29–31), ficin (18), papain (32, 33), and chymopapain (34, 35). Although there is disagreement, the studies generally conclude that commercial DFP inhibits cysteine proteases but that the inhibition of the active site sulfhydryl is due to an undefined impurity in most commercial preparations. Purified DFP phosphorylates serine (18, 35) or tyrosine (31, 32) is residual in these proteins without inhibiting their protease ac-

tivity (18, 30–32, 35), and the DFP impurity irreversibly inhibits the active site sulfhydryl (18, 31). Since the evidence presented here suggested cancer procoagulant was a cysteine protease, it seemed likely that the inhibition by DFP, a consistent and reproducible observation (9, 10, 14), was probably due to impurities in the commercial DFP preparations that were employed. To confirm this hypothesis, DFP was catalytically hydrolyzed to diisopropylphosphate and hydrofluoric acid. The conjectured DFP impurity is not hydrolyzed under these conditions, so that during the course of the reaction inhibition of serine proteases should decrease while inhibition of cysteine proteases should remain unchanged (18). Inhibition of thrombin activity was almost complete when treated with the initial (0 min) DFP hydrolyzate mixture, but after 2 h the DFP hydrolyzate had lost virtually all of its ability to inhibit the clotting activity of this serine protease. In contrast, the inhibition of cancer procoagulant by the DFP hydrolyzate remained unchanged during the 2 h hydrolysis of DFP. Thus, all the data are consistent with the hypothesis that cancer procoagulant is a cysteine protease and its inhibition by DFP is due to an impurity in the Aldrich DFP used in this research.

DFP inhibition of cancer procoagulant activity was reversed by reductive reactivation with DTT. Although other investigators suggest that inhibition of cysteine proteases by the DFP impurity is essentially irreversible (18, 29, 31), their data show partial (~10%) reactivation of bromelain with 5 mM cysteine (29) and about 40% reactivation of ficin with 0.25 M cysteine (18). Our use of DTT, a more potent reducing agent than cysteine, different reactivation conditions, and the differences in the cysteine proteases may account for our more effective reactivation of DFP-inhibited cancer procoagulant.

Russell's viper venom standard was used to compare the effect of these inhibitors on a serine protease that activates Factor X in a well-characterized manner (27, 36–38). Mercury (0.1 mM) had little or no effect on the procoagulant activity of Russell's viper venom, which is consistent with the observations of others (38); and 1 mM iodoacetamide seemed to stimulate the activity of Russell's viper venom. Thus, there was a clear distinction between the effect of these inhibitors on the activity of cancer procoagulant and the serine protease in Russell's viper venom. These data strongly suggest that cancer procoagulant is a cysteine protease, but unequivocal evidence from an amino acid analysis demonstrating a chemically modified active site cysteine must await the purification of milligram amounts of the enzyme.

Several groups have suggested that malignant cells produce higher levels and possibly different types of cysteine proteases than normal cells (39, 40). Evidence from several laboratories has suggested that malignant

cells produce higher levels of "thromboplastic" coagulation factors than normal cells (41–43), but clear definition of these factors has been difficult to obtain. Although there is evidence that suggests that malignant cells produce an abnormal coagulation activity that is not associated with normal cells (9–13),⁶ it has not been clear whether this is a known coagulation factor or whether it is something unique to the malignant state. This study suggests that a cysteine protease extracted from rabbit V2 carcinoma activates Factor X, an observation that distinguishes it from other coagulation enzymes.

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