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A CYCLOPEPTIDIC SUICIDE SUBSTRATE PREFERENTIALLY INACTIVATES UROKINASE-TYPE PLASMINOGEN ACTIVATOR

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c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] was designed and studied as a mechanism-based inactivator (suicide substrate) for plasminogen activators (u-PA and t-PA) and plasmin. This compound inhibited u-PA and fulfills criteria expected for the involvement of an enzyme-activated inhibitor : first-order and irreversible process, saturation kinetics, protection by substrate. The limiting first-order rate constant k_{inact} and the apparent enzyme-inhibitor dissociation constant K_I were 0.021 s^{-1} and 9 µM, respectively at pH 7.5 and 25 °C. The activation of plasminogen by u-PA is compromised after this enzyme has been treated by the reagent. Plasmin and t-PA were inactivated 40- and 2330-fold less efficiently than u-PA, respectively.

Plasmin (EC 3.4. 21.7), a serine protease with a broad specificity is generated from the enzymatically inactive zymogen plasminogen by action of two immunologically unrelated plasminogen activators (EC 3.4.21.31), tissue plasminogen activator (t-PA) and urokinase (u-PA). In addition to fibrinolysis (1), plasminogen activation is associated with a number of physiological and pathological processes like angiogenesis (2), cell migration (3), embryonic implantation (4) and tumor cell metastasis (5). A correlation has been shown between the expression of tumor cell u-PA and tumor invasion (5-8). Specific synthetic inhibitors of plasminogen activators, by inhibiting the initial protease responsible for plasmin generation, are of interest in different applications. They include analysis of their biological roles and potential therapeutic uses through the control of extracellular proteolysis. Few synthetic inactivators of these enzymes are known, among them peptidic derivatives of lysyl or arginyl chloromethylketones (9, 10) acting by affinity labeling and halomethylated derivatives of 3,4-dihydrocoumarins (11, 12) behaving as mechanism-based inactivators (suicide substrates) (13-15). A selective alkylation of the active site histidine of high-molecular-weight urokinase by a dihydrocoumarin derivative was demonstrated (14).

This paper presents a further approach to mechanism-based inhibition of plasminogen activators using a novel inhibitor, c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] (Fig. 1, aB =

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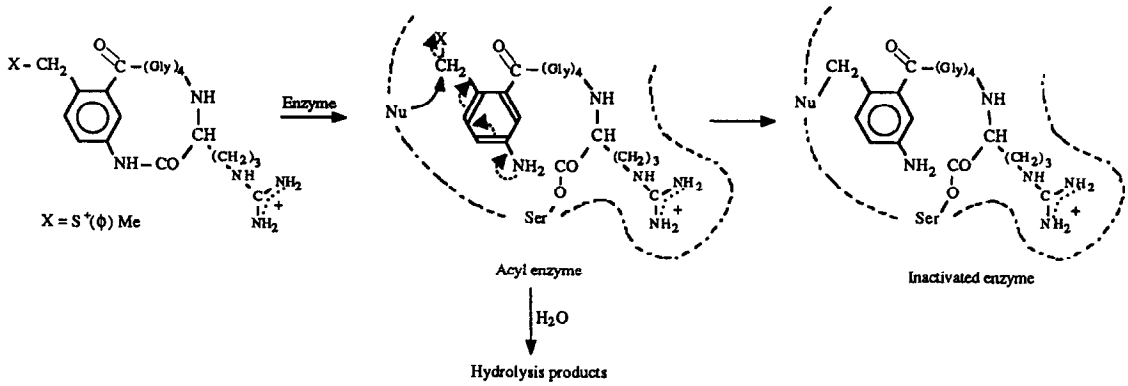

Figure 1. Postulated mechanism for the inactivation of plasminogen activators and plasmin by c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$].

3-aminobenzoic acid residue). This inhibitor is a member of a new family of suicide substrates which has been designed by including a latent electrophilic quinonimmonium methide (16, 17) into a cyclopeptide ring which amino acid sequence dictates specificity (18). Its presumed mode of action through the formation of an acyl-enzyme with the simultaneous unmasking of the electrophilic function is based upon previous findings in the dihydrocoumarin series (11, 12, 19, 20). Other functionalized cyclopeptides have been targeted against proteases of chymotrypsic specificity and act as enzyme inactivators (18, 21). The NMR conformational analysis of c[Arg-aB-(CH_3)-Gly_4] has demonstrated the conformational flexibility of the peptidic backbone (22). We now report the salient features of the inactivation of both plasminogen activators and plasmin by c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$], a potential suicide inhibitor possessing a latent thioanisole leaving group; its influence on the fibrinolytic activity of urokinase is also analyzed.

MATERIALS AND METHODS

Human urinary urokinase (RFU 203) was obtained from Choay. Based on quantitative gels, this preparation was more than 95 % pure in the high-molecular-weight form. Human plasmin was purchased from Kabi Vitrum, France. Two-chain t-PA, obtained from cultured melanoma cells, was purchased from Biopool, Sweden. Its concentration in solution was determined by amino acid analysis. Active-site titrations were carried out with 4-methyl-umbelliferyl-*p*-guanidinobenzoate for urokinase (23) and *p*-nitro-*p'*-guanidinobenzoate for plasmin (24). The chromogenic substrates L-pyroglutamyl-L-glycyl-L-arginine-pNA (S-2444) of urokinase, D-isoleucyl-L-prolyl-L-arginine-pNA (S-2288) for t-PA and D-valyl-L-leucyl-L-lysine-pNA (S-2251) of plasmin were from Kabi Vitrum. The synthesis of c[Arg-*m*-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] will be reported elsewhere.

Enzymic activity assays. The amidolytic activity of urokinase was determined towards S-2444 (100 µM) in 0.025 M phosphate, 0.1 M NaCl, 0.05 % (v/v) Tween 80 at pH 7.5 and 25 °C by measuring the production of nitroaniline at 405 nm using a Lambda 5 Perkin Elmer spectrophotometer. Plasmin was assayed similarly with 280 µM S-2251 in 0.1 M sodium phosphate, 25 % (v/v) glycerol at pH 7.5 and 25 °C. t-PA was assayed with 280 µM S-2288 in 0.05 M Tris, 0.1 M NaCl, 0.01 % Tween 80 at pH 7.5.

Enzyme inactivation (incubation method). Urokinase (0.55 µM) was incubated with various concentrations of inhibitor (2.75-41.2 µM). Aliquots (10 µl) were withdrawn with time and their residual activity was determined in standard conditions after filtration-centrifugation at 4 °C on Centricon 30 microconcentrator to remove the inhibitor. The apparent pseudo-first-order constants

for inactivation, k_{obs}, were obtained from least-squares analysis of semilog plots of percentage of remaining activity against time. This method was also used to analyze plasmin inhibition with [E] = 1.9 µM, [I] = 34-112 µM and t-PA inhibition with [E] =0.75 µM, [I] = 0.37-1 mM at pH 7.5 and 25 °C. The buffers were identical to that used in the activity assays. In some cases, the filtered enzymes were treated for 30 min by 0.75 M NH_2OH at pH 8.5, 25 °C and filtered again before determination of activity. For the determination of the partition ratio, incubations with urokinase (0.55 µM) were carried out in duplicate as described above, using inhibitor concentrations ranging from 0.89 to 11 µM. Enzyme activity towards S-2444 was determined 18 h after the addition of the inhibitor relative to a blank that did not contain inhibitor.

Enzyme inactivation in the presence of the substrate (progress curve method). Urokinase inhibition was assayed by progress curve analysis according to Hart and O'Brien (25) in the following experimental conditions : [E] = 31 nM; [S-2444] = 75 µM; [I] = 5-21.9 µM; pH 7.5 and 25 °C ([I] = 0 in the reference cell). The kinetics were treated according to the scheme shown in eq. 1. Species E, I, E*I, E-I, ES and P represent, respectively, enzyme, inhibitor, enzyme-

$$\begin{array}{ccccc} & & K_I & & k_{inact} \\ & + I & \rightleftharpoons & E^*I & \longrightarrow & E\text{-}I \\ E & & & & & \\ & + S & \rightleftharpoons & ES & \longrightarrow & E + P \\ & & K_S & & k_c & \end{array} \qquad \text{(eq. 1)}$$

inhibitor complex formed prior to k_{inact}, inhibited enzyme with the inhibitor covalently attached, enzyme-substrate (S-2444) complex, hydrolysis product of S-2444. The simplified inactivation scheme (line 1, eq. 1) is derived from a more complete scheme that includes the acylation step (eq. 2) where EI is the Michaelis complex and E~I the acyl-enzyme. The expression in eq. 1 (line 1) is obtained

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI \xrightarrow{k_2} E\sim I \xrightarrow{k_3} E\text{-}I \qquad \text{(eq. 2)}$$

by combining k_2 and k_3. The rate v of change in absorbance at 405 nm due to the hydrolysis of S-2444 was obtained from computer-assisted spectrophotometer. A plot of ln v *versus* time gives a straight line characterized by a slope of $-\pi$. The analysis by statistical treatment of Wilkinson (26) of the plot $1/\pi$ *versus* $1/[I](1-\alpha)$, with $\alpha = [S]/K_M+[S]$, (eq. 3), allows the determination of K_I and k_{inact}, the apparent enzyme-inhibitor dissociation constant and the inactivation rate constant at infinite inhibitor concentration, respectively.

$$1 / \pi = K_I / k_{inact} [I] (1-\alpha) + 1 / k_{inact} \qquad \text{(eq. 3)}$$

Fibrinolysis assay. After treatment of urokinase (47 nM) by the inhibitor (0.88-7.95 µM) during 30 min at pH 7 (25 mM sodium phosphate, 0.1 M NaCl, 0.05 % Tween 80), and removal of the inhibitor (Centricon 30), the plasminogen-activating properties of the treated enzyme was evaluated by fibrin clot lysis experiments. ^{125}I-labeled fibrin clots were prepared as previously described (15) and incubated at 37 ° C in 200 µl of enzyme sample. The extent of fibrinolysis upon addition of urokinase previously treated by the inhibitor (final concentration 1.6 nM; no inhibitor for the blank) was calculated at 30 min intervals from the amounts of radioactivity released from the clot into supernatant after the digestion of the clot had been stopped by adding cold 0.15 M NaCl. Experiments were performed in duplicate.

RESULTS

Inactivation kinetics. c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] is a time-dependent inactivator of urokinase, t-PA and plasmin (Figure 2). For all enzymes, no reactivation (< 1 %) was observed after elimination by filtration (Centricon 30) of the reagent and incubation at 4 ° C for 16 h. In the same conditions, the spontaneous loss of activity of a control sample was < 5%. Addition of buffered hydroxylamine (0.75 M, pH 8.5, 25 °C) to the inhibited enzymes resulted in less than 2 % reactivation. The activity losses followed pseudo-first-order kinetics characterized by the rate constants k_{obs}. The second-order rate constants $k_{obs}/[I]$ for the inactivation of plasmin and t-PA were 40 and 1 $M^{-1}s^{-1}$, respectively (Table I).

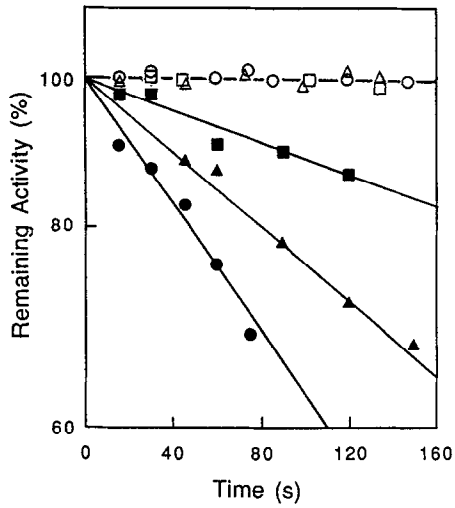

Figure 2. Kinetics of inactivation of urokinase (● , 0.55 μM), t-PA (■ , 0.75 μM) and plasmin (▲ , 1.9 μM) by c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] (2.75, 1000 and 67 μM, respectively) at pH 7.5 and 25 °C. Corresponding blanks : (○ , □ , △ , respectively).

Inactivation rates for urokinase were too fast to be measured accurately under first-order conditions. Therefore the kinetic parameters were determined in the presence of the substrate S-2444 by progress curve analysis (25) (Figure 3, Table I). Using the titration method (28), the partition ratio k_{cat}/k_{inact} for the inactivation of urokinase was determined from the intercept with the *x* axis *minus* 1 of the linear plot of the fraction of enzyme activity $[E]/[E]_o$ at infinite time *versus* the molar excess of inhibitor over enzyme $[I]_o/[E]_o$ and was found equal to 4 (Figure 4). This ratio represents the average number of enzyme turnovers per inactivation event since k_{cat} is the turnover rate constant for the hydrolysis of the inhibitor catalyzed by the enzyme. From the partition ratio, k_{cat} was evaluated at 0.084 s^{-1}.

Substrate protection. Increasing amounts of substrate S-2444 at fixed inhibitor concentration (progress curves) protect urokinase against inactivation. This was confirmed

TABLE 1. Kinetic constants for the inactivation of high-molecular-weight urokinase, two-chain t-PA and plasmin by c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] (I) (pH 7.5 and 25 °C) and 3-benzyl-6-chloromethyl-3,4-dihydrocoumarin (II)

Inhibitor	Urokinase			t-PA	Plasmin
	K_I (M)	k_{inact} (s^{-1})	k_{inact}/K_I ($M^{-1}s^{-1}$)	k_{inact}/K_I ($M^{-1}s^{-1}$)	k_{inact}/K_I ($M^{-1}s^{-1}$)
I	9.0×10^{-6} [a]	0.021[b]	2 330	~1[c]	40[c]
II[d]			2 100	187	136

[a] ± 1.4×10^{-6} [b] ± 0.002 [c] k_{inact}/K_I was obtained as $k_{obs}/[I]$ at low concentrations of inhibitors (27) [d] Data from references 14 (urokinase, pH 6.8, 4 °C) and 15 (t-PA, pH 6.8, 4 °C; plasmin, pH 7.3, 4 °C).

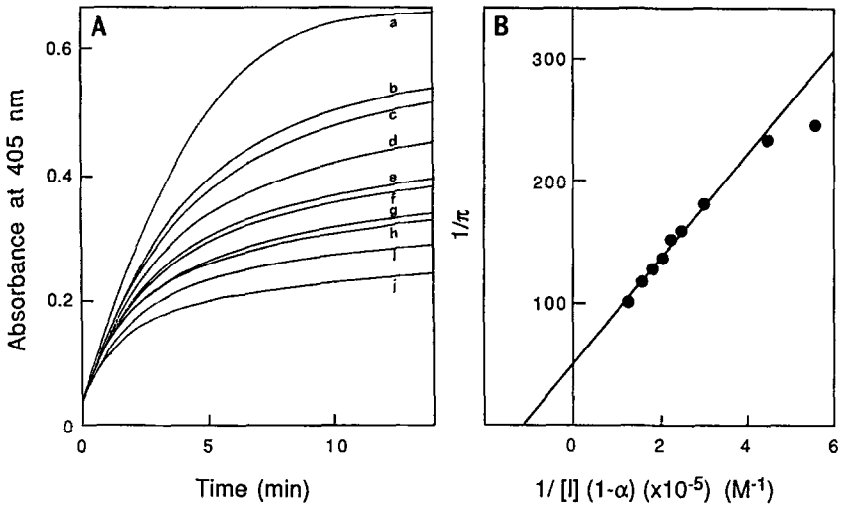

Figure 3. A.Time course of hydrolysis of S-2444 (75 μM) by urokinase (31 nM) in the absence (a) and in the presence of various concentrations of c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] : (b) 5, (c) 6.25, (d) 9.37, (e) 11.2, (f) 12.5, (g) 13.7, (h) 15.6, (i) 17.5, (j) 21.9 μM at pH 7.5 and 25°C. B. Determination of K_I and k_{inact}.

using the incubation method. Addition of substrate to the urokinase incubation mixture resulted in a significant decrease in the inactivation rate constant. The rate constant $k_{obs}/[I]$ for the inactivation of urokinase (0.66 μM) by the inhibitor (4 μM) in the presence of 260 μM S-2444 was decreased by a factor of 1.6 compared to that obtained in the absence of S-2444.

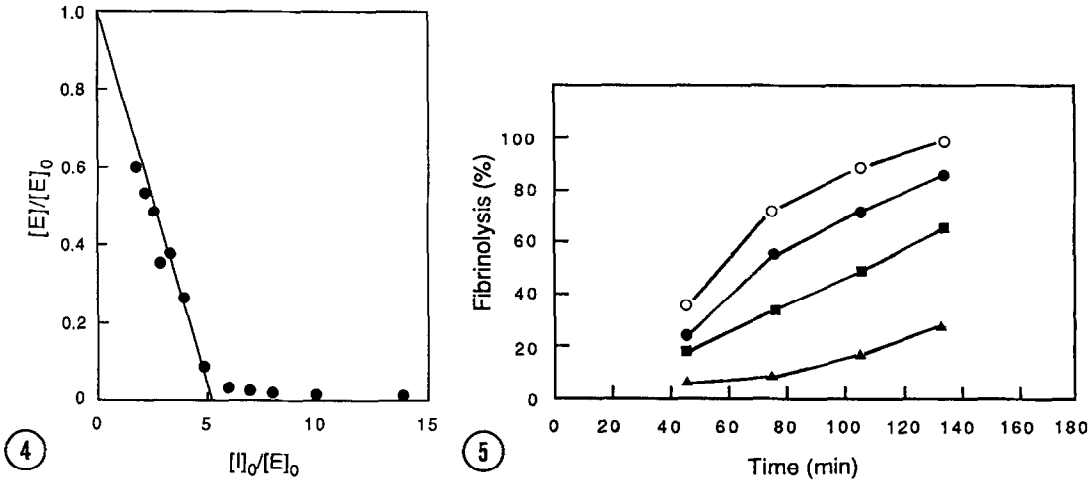

Figure 4. Determination of the partition ratio for the inactivation of urokinase by c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] (pH 7.5 and 25 °C) : plot of the fraction of remaining activity *versus* $[I]_0/[E]_0$.

Figure 5. Lysis of ^{125}I-labeled fibrin clots by urokinase (final concentration 1.6 nM) before (○) and after previous treatment of urokinase (47 nM) by c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$]: 0.88 (●), 2.65 (■) and 7.95 (▲) μM.

Inhibition of fibrin clot lysis induced by urokinase. Figure 5 shows that the dissolution of ^{125}I-labelled fibrin clots induced by urokinase is prevented in a dose-dependent manner when the enzyme has been previously treated by various amounts of inhibitor over enzyme ($[I]_0$ /$[E]_0$ =19-170). Complete lysis of clots gives the 100 % value.

DISCUSSION

In this work, the differential interaction with human high-molecular-weight urokinase, two-chain t-PA and plasmin of a new potential suicide substrate c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] was examined and can be discussed as follows : (a) characteristics of the enzyme-mediated process of inhibition; (b) selectivity and efficiency of the inhibitor compared to other known inactivators. In the presence of urokinase, t-PA and plasmin, a time- and concentration-dependent inhibition was observed with no significant spontaneous recovery of activity. The lack of reactivation after treatment by hydroxylamine excludes the formation of a stable acyl-enzyme and indicates that the enzyme alkylation is the major pathway. First-order kinetics are observed for the inactivation process. The kinetic results obtained for urokinase fit to the minimal scheme reported above (eq. 1, line 1). A synthetic substrate protects urokinase against inactivation by the reagent indicating that the chemical modification occurs at the active site. Consequently, criteria expected for mechanism-based inactivation (28) are met supporting the postulated mechanism described in Figure 1. In the transient acyl-enzyme, the reactive *p*-aminobenzyl derivative may be attacked by a nucleophilic enzymic residue, thus leading to covalent modification and enzyme inactivation. Alternatively, the enzyme activity may be restored after water-mediated deacylation of the acyl-enzyme. A good partition ratio (equal to 4) is observed for urokinase. Partition ratios ranging from 1.7 to 91 have been reported for the inactivation of a serine protease (chymotrypsin) by haloenol lactones (29).

c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] is found to be a good inactivator of urokinase acting in the same range of efficiency that 3-benzyl-6-chloromethyl-3,4-dihydrocoumarin (Table 1). This also holds for the inhibition of the fibrinolytic activity of urokinase by the cyclopeptide (Figure 5). A molar excess of inhibitor over enzyme of 56 leads to 50 % fibrinolysis after a period of 120 min. The same effect was observed with one dihydrocoumarin derivative with $[I]_0/[E]_0$ = 11. Interestingly, a better discrimination between urokinase and plasmin is observed with the cyclopeptidic inactivator (Table 1) : the inactivation potency k_{inact}/K_I is higher for urokinase than for plasmin by a factor of 15 (dihydrocoumarin derivative) and a factor of 76 (cyclopeptide). Among plasminogen activators, the inactivator inhibits specifically urokinase with no significant inactivation of t-PA. Some tripeptide chloromethylketones has been described as efficient inactivators of urokinase (9, 10, 30). Glu-Gly-Arg-CH_2Cl inactivates urokinase 18-fold faster than t-PA but the efficiency for the inactivation of t-PA is not negligible (402 M^{-1} s^{-1}) compared to the cyclopeptide (1

$M^{-1}s^{-1}$). In contrast to the chloromethylketones affinity labels, c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] is essentially chemically inert until the enzyme-catalyzed activation occurs leading to production of the reactive form of the inhibitor within the active site. A renal toxicity has been reported for a peptide chloromethylketone able to inhibit neutrophil elastase (31).

The two main features displayed by c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$], specific inactivation of urokinase (compared to t-PA) and alteration of the kinetic parameters of the modified urokinase for the activation of plasminogen, are of interest in experiments aiming to establish the differential role of u-PA and t-PA in physiological processes like the movement of cells and the biology of reproduction. Many observations suggest an *in vivo* role for u-PA as an activator in cell migration and matrix degradation under normal and also pathological circumstances (32, 33). An u-PA-plasmin-collagenase cascade implicating the plasmin-mediated activation of procollagenases is probably a major mechanism enabling cells to degrade surrounding structures (34). The balance between PA and their natural inhibitors determines the degree of proteolytic activity in the pericellular area. Three genetically and immunogenically distinct types of natural inhibitors (PAI 1, PAI 2 and protease nexin) have been described (35). It has been demonstrated that the selective inhibition of cancer cell u-PA by PAI 2 prevents the plasminogen-dependent degradation of labeled extracellular matrix (36). Antibodies were also used to inhibit invasion by metastatic cells (5, 37). Consequently, a specific synthetic inhibitor of u-PA like c[Arg-aB-($CH_2\overset{+}{S}CH_3\phi$)-$Gly_4$] is interesting to consider as a potential drug to overcome an u-PA-natural inhibitor imbalance in pathological situations.

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REFERENCES

1. Rijken, D. C., Hoylaerts, M. and Collen, D. (1982) J. Biol. Chem. 257, 2920-2925.
2. Gross, J. L., Moscatelli, D. and Rifkin, D. B. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2623-2627.
3. Ossowski, L., Quigley, J. and Reich, E. (1979) In Proteases and Biological Control (E. Reich, D. B. Rifkin and E. Shaw, Ed.) PP. 901-913. Cold Spring Harbor, New York.
4. Strickland, S. E., Reich, E. and Sherman, M. I. (1976) Cell, 9, 231-240.
5. Ossowski, L. and Reich, E. (1983) Cell, 35, 611-619.
6. Ossowski, L. (1988) Cell, 52, 321-328.
7. Hearing, V. L., Law, L. W., Corti, A., Appella, E. and Blasi, F. (1988) Cancer Res. 1270-1278.
8. Brunner, G., Pohl, J., Erkell, L. J., Radler-Pohl, A. and Schirrmacher, V. (1989) J. Cancer Res. Clin. Oncol. 115, 139-144.
9. Kettner, C. and Shaw, E. (1979) Biochim. Biophys. Acta, 569, 31-40.
10. Lijnen, H. R., Van Hoef, B. and Collen, D. (1987) Eur. J. Biochem. 162, 351-356.
11. Béchet, J.-J., Dupaix, A., Yon, J., Wakselman, M., Robert, J.-C. and Vilkas, M. (1973) Eur. J. Biochem., 35, 527-539.
12. Nicolle, J. P., Hamon, J. F. and Wakselman, M. (1977) Bull. Soc. Chim. Fr., 83-88.

13. Reboud-Ravaux, M., Desvages, G. and Chapeville, F. (1982) FEBS Lett., 140, 58-62.
14. Reboud-Ravaux, M. and Desvages, G. (1984) Biochim. Biophys. Acta, 791, 333-341.
15. Mor, A., Maillard, J., Favreau, C. and Reboud-Ravaux, M. (1990) Biochim. Biophys. Acta, 119-127.
16. Wakselman, M. (1973) Nouv. J. Chimie, 7, 439-447.
17. Decodts, G. and Wakselman, M. (1983) Eur. J. Med. Chem., 18, 107-111.
18. Wakselman, M., Mazaleyrat, J.-P., Xie, J., Montagne, J.-J., Vilain, A.-C. and Reboud-Ravaux, M. (1991) Eur. J. Med. Chem., in press.
19. Béchet, J.-J., Dupaix, A., Roucous, C. and Bonamy, A. M. (1977) Biochimie, 59, 241-246.
20. Vilain, A. C., Okochi, V., Vergely, I., Reboud-Ravaux, M., Mazaleyrat, J.-P. and Wakselman, M. (1991) Biochim. Biophys. Acta, 1076, 401-405.
21. Mazaleyrat, J.-P., Reboud-Ravaux, M. and Wakselman, M. (1987) Int. J. Pept. Prot. Res., 30, 622-633.
22. Convert, O., Mazaleyrat, J.-P., Wakselman, M., Morize, I. and Reboud-Ravaux, M. (1990) Biopolymers, 30, 583-591.
23. Jameson, G. W., Roberts, D. V., Adams, R. W., Kyle, W. S. A. and Elmore, D.T. (1973) Biochem. J. 131, 107-117.
24. Chase, T. and Shaw, E. (1970) Methods Enzymol. 19, 20-27.
25. Hart, G. J. and O'Brien R. D. (1973) Biochemistry, 12, 2940-2945.
26. Wilkinson, G. N. (1961) Biochem. J., 80, 324-332.
27. Kitz, R. and Wilson, I. B. (1962) J. Biol. Chem. 237, 3245-3249.
28. Silverman, R. B. (1988) In Mechanism-Based Enzyme Inactivation : Chemistry and Enzymology, Vol. 1, pp. 3-30, CRC, Boca Raton.
29. Daniels, S. B., Cooney, E., Sofia, M. J., Chakravarty, P. K. and Katzenellenbogen, J. A. (1983) J. Biol. Chem. 258, 15046-15053.
30. Lijnen, H. R., Uytterhoeven, B. and Collen, D. (1984) Thromb. Res., 34, 431-437.
31. Ranga, V., Kleinerman, J., Ip, M. P. C., Sorensen, J. and Powers, J. C. (1981) Am. Rev. Respir. Dis. 124, 613-618.
32. Saksela, O. and Rifin, D. B. (1988) Ann. Rev. Cell Biol. 4, 93-126.
33. Reboud-Ravaux, M. (1985) Biochimie, 67, 11097-12012.
34. Moscatelli, D. and Rifkin, D. B. (1988) Biochim. Biophys. Acta, 948- 67-85.
35. Kruithof, E. K. O. (1988) Enzyme 40, 113-121.
36. Baker, M. S., Bleakley, P., Woodrow, G. C. and Doe, W. F. (1990) Cancer Res. 50, 4676-4684.
37. Reich, R., Thompson, E. W., Iwamoto, Y., Martin, G. R., Deason, J. R., Fuller, G. C. and Miskin, R. (1988) Cancer Res., 48, 3307-3312.