thawing hemolysis gave the same range of values as sonication, except in the case of hexokinase at both temperatures and of lactate dehydrogenase at 25 °C. Comparison of our results with the highest activities found in the literature[1–5] shows that sonication accompanied by modification of the test composition leads to better yields in the case of many glycolytic activities. As shown in Table II, phosphofructokinase, glyceraldehyde-3-P dehydrogenase, 3-P-glycerate kinase and lactate dehydrogenase tested at 25 °C were significantly higher than reported by LÖHR and WALLER[2,3]. A statistical assessment of the activities at 37 °C was not possible, because the corresponding RAPOPORT[4] data do not contain standard deviation and number of sample values. However, our activities were higher by 40% or more in the case of hexokinase, phosphofructokinase, aldolase, glyceraldehyde-3-P dehydrogenase and 3-P-glycerate kinase[8].

Zusammenfassung. Die Aktivitäten von 7 glykolytischen Enzymen wurden in normalen menschlichen Erythrozyten gemessen. Kurze Ultraschall-Hämolyse und modifizierte Test-Bedingungen führten, verglichen mit den höchsten Werten, zu signifikant höheren Aktivitäten.

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Specificity of Potato Kallikrein Inhibitors for Kallikreins

Potato kallikrein inhibitor has been initially discovered by WERLE et al.[1] and partially purified on this material[2,3]. However, details have never been elucidated on the intrinsic material, much remaining obscure. In the present paper, inhibitors, acting specifically on human plasma kallikrein, were discovered in potatoes and studied as to their purification and properties, including their inhibitory specificity against many kinds of kallikreins or protease enzymes. Our final purpose was to clarify the pathological roles or meanings of kinin liberation in blood by means of development of such specific inhibitors.

Sixteen kinds of Japanese and 22 foreign potatoes were examined to screen their contents. Potato kallikrein inhibitors (PKI) were found to contain 60–190 kallikrein inhibitor units (KIU) per g in various kinds of potatoes. The Japanese potato '*Danshaku-Imo*' was mainly used for our study and contained about 140 KIU per 1 g of this fresh potato. Purification of PKI was performed by the combination of salting out with ammonium sulphate, dialysis against water, DEAE-cellulose treatment, chromatography on columns of CM-cellulose, CM-Sephadex and hydroxyapatite, and the 'Ampholine' electrofocusing method[4]. Two kinds of inhibitors were found in potatoes and their isoelectric points were pH 5.6 and pH 6.4. Both were isolated in homogenous form, checked by disc-electrophoresis and ultracentrifugation. Molecular weights of these inhibitors (pI 5.6 and pI 6.4) were measured as follows:

Inhibitors	Ultra-centrifugation	Gel-filtration (Sephadex G-100)	Calculation from amino acid analysis
pI 5.6	24,200	25,000	23,388
pI 6.4	28,700	26,000	22,814

Both were readily soluble in neutral saline solution and unstable on heating.

Each 2 inhibitors were pre-incubated with various proteases and the inhibitory activities at less than 50% inhibition were measured on the dog vasodilator[5], esterolytic[6,7], caseinolytic[8] and fibrinolytic[9] activities (Table) and compared with that of Trasylol (bovine lung kallikrein – trypsin – inhibitor). Purified PKI inhibited human plasma kallikrein (activated with acetone) strongly, hog pancreatic kallikrein and human plasmin (streptokinase activated euglobulin) only slightly and bovine trypsin and α-chymotrypsin also slightly, while Trasylol has broadly strong actions for hog pancreatic kallikrein, human plasmin and bovine trypsin, and slight action for human

Inhibitory effects of potato kallikrein inhibitors and trasylol

Enzymes	Method or substrate	PKI pI 5.6	PKI pI 6.4	Trasylol
Human plasma kallikrein	dog assay[5]	24,800	24,000	9,700
Hog pancreatic kallikrein	dog assay[5]	3,680	9,000	46,000
	BAEE[6]	208	313	12,025
Trypsin	casein[8]	11	11	51
	BAEE[6]	240	280	400
α-Chymotrypsin	casein[8]	25	40	47
	BTEE[7]	140	110	142
Human plasmin	fibrinolysis[9]	229[a]	246[a]	43,100[a]

Numbers are inhibited units per μmole of inhibitors, Frey units by dog assay, Kunitz's units by caseinolysis and μmoles of substrate (BAEE, BTEE) hydrolyzed per min. Inhibitory units per μmole of inhibitors are expressed as based on the molecular weights of PKI, 25,000 (assumed), and trasylol, 6500. [a] Inhibited human euglobulin equivalent to the original plasma volume (ml/μmole of inhibitors). BAEE, N^{α}-benzoyl-L-arginine ethylester; BTEE, benzoyl-L-tyrosine ethylester.

[1] E. WERLE, L. MAIER and F. LÖFFLER, Biochem. Z. *321*, 372 (1951).
[2] E. WERLE and L. MAIER, Biochem. Z. *322*, 414 (1952).
[3] E. WERLE, W. APPEL and E. HAPP, Z. Vitamin-, Hormon-, Fermentforsch. *10*, 127 (1959).
[4] O. VESTEBERG and H. SVENSSON, Acta chem. scand. *20*, 820 (1966).
[5] H. MORIYA, K. YAMAZAKI and H. FUKUSHIMA, J. Biochem., Japan *58*, 201 (1965).
[6] G. W. SCHWERT and Y. TAKENAKA, Biochim. biophys. Acta *16*, 570 (1955).
[7] B. C. W. HUMMEL, Can. J. Biochem. Physiol. *37*, 1393 (1959).
[8] M. KUNITZ, J. gen. Physiol. *30*, 291 (1947).
[9] M. LASSEN, Acta physiol. scand. *27*, 371 (1952).

plasma kallikrein. It was found that both PKI had only weak inhibitory actions, 250–1250 KIU per μmole of PKI, against human salivary and urinary kallikreins. From these results, PKI seems to be one of the polyvalent protease inhibitors, especially specific and some typical inhibitors for human plasma kallikrein.

Also they were about 2.5 times stronger than soybean trypsin inhibitor (SBTI, 2 times crystallized) which is a typical plasma kallikrein inhibitor, against human plasma kallikrein. However, they did not inhibit *Seratia* proteinase and pronase-P, and were inactivated by digestion of pepsin, papain and *Seratia* proteinase. Detailed differences in inhibitory specificity between 2 inhibitors (pI 5.6 and pI 6.4) are now under investigation.

It was recognized that the inhibitors had potent anti-inflammatory actions in rats with carrageenin edema and CM-cellulose pouch exudate by reducing edema strength

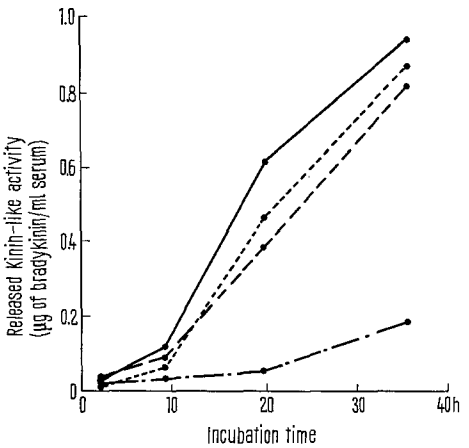

Fig. 1. Inhibitory effect of protease inhibitors on liberation of kinin-like substance from acidified rat serum in vitro. Rat serum was acidified to pH 4.0 with acetic acid and incubated with protease inhibitors at 37 °C. Released kinin-like activity was checked by dog assay. ————, control; – – – – – –, Trasylol, 1034 KIU/ml serum; — — —, soybean trypsin inhibitor, 1.034 mg/ml serum; — – — –, potato kallikrein inhibitors, 1051 KIU/ml serum.

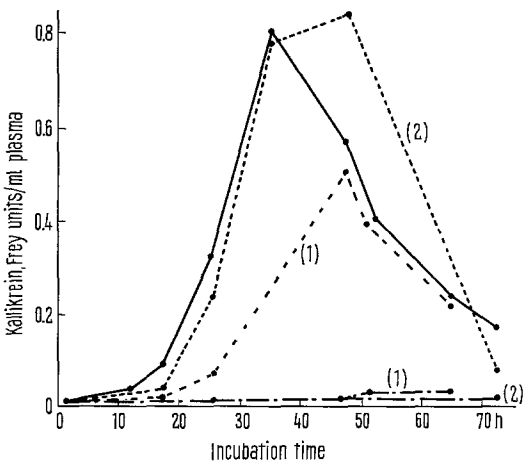

Fig. 2. Inhibitory action of protease inhibitors on liberation of activated plasma kallikrein by acidification of human plasma in vitro. Human plasma was adjusted to pH 5.0 with hydrochloric acid and incubated with protease inhibitors at 37 °C. Liberated kallikrein was measured by dog assay. ————, control; – – – – –, Trasylol, 1) 64 KIU/ml plasma, 2) 10 KIU/ml plasma; — – — –, potato kallikrein inhibitors, 1) 64 KIU/ml plasma, 2) 10 KIU/ml plasma.

(85% inhibition statistically significant at p 0.05, 2 h after i.p. injection of the partially purified inhibitor both mixed, 1 mg/100 g body weight) and exudations of leucocytes (82% inhibition after 2 h i.m. injection, same dosage) and proteins (19% inhibition after 6 h i.m. injection, same dosage) respectively, but they had no action on egg white edema which is probably induced by histamine. They inhibited the formation of the kinin-like substances with edema-producing activity from rat serum when acidified in vitro, especially by acetic acid[10] (Figure 1) and also inhibited plasma kallikrein activated just from human plasma by acidification in vitro (Figure 2). Such inhibitory actions on the kallikrein-kinin system were much stronger than Trasylol and even soybean trypsin inhibitor (Figures 1 and 2). These results indicate that kinin(s) released by plasma kallikrein may act as a mediator in some inflammatory states and that the inhibitor blocked the reaction to some extent.

In potato, various protease inhibitors which seemed to be proteins or peptides, had been reported, but their relationship or detailed information have remained obscure. From the results we examined on potato chymotrypsin inhibitor I, kindly supplied from Dr. C. A. RYAN[11], it had no inhibitory action against both human plasma and hog pancreatic kallikreins, while it showed more than 10 times stronger action than PKI against human salivary and urinary kallikreins. PKI was clearly recognized as different from this chymotrypsin inhibitor I from these behaviours, and also observed to be different from the polyvalent protease inhibitors in potato recently reported by K. HOCHSTRASSER et al.[12], in which structural amino acids composition was not consistent with that of our PKI, namely while PKI contained methionine and less half-cystine, inhibitors isolated by HOCHSTRASSER contained no methionine and fairly more half-cystine. As for other inhibitors isolated from potato, some of their properties were different from those of PKI, especially as to heat stability, purification methods, and so on; therefore our PKI examined in this study must be new inhibitors.

We are interested in the possible selective actions of these inhibitors on various kallikreins and proteolytic enzymes, and would like to elucidate the pathological roles of kinins by means of using such specific inhibitors of kinin-liberation. Investigations are now being continued and detailed papers will be published in the near future.

Summary. Two polyvalent protease inhibitors, acting especially on plasma kallikrein, were isolated from potatoes. Some of their physicochemical and biological properties were investigated.

Zusammenfassung. Aus Kartoffeln wurden 2 polyvalente Proteaseninhibitoren im besonderen für Plasmakallikrein isoliert. Einige physikochemische und biologische Eigenschaften der Inhibitoren wurden charakterisiert.

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[10] K. TAKAGI and S. KAYAOKA, Folia pharmac. jap. *64*, 14 (1958).
[11] C. A. RYAN, Biochemistry *5*, 1592 (1966).
[12] K. HOCHSTRASSER, E. WERLE, R. SIEGELMANN and S. SCHWARZ, Hoppe-Seyler's Z. physiol. Chem. *350*, 897 (1969).