*Br. J. Pharmac. Chemother.* (1967), **30**, 425–435.

# CHARACTERIZATION OF A PLASMA KININ-INACTIVATING ENZYME PRODUCED BY *PSEUDOMONAS AERUGINOSA*

BY

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*(Received March 6, 1967)*

The occurrence of a kinin-inactivating enzyme, a kininase, in the growth medium of *Psuedomonas aeruginosa* (*Ps. aer.*) has been reported in a previous paper (Rugstad, 1966), where the relationship between the growth of this microbe and the amount of kininase produced was also discussed. The purification of this kininase and some of its properties has been described subsequently (Rugstad, 1967). The kinins have often been mentioned as possible mediators in the inflammatory response, and it is tempting to speculate whether the ability of *Ps. aer.* to eliminate kinins may be related to its pathogenicity. Not only the amount but also the type of kininase activity present may be of importance in this connexion. More detailed knowledge about the properties of this kininase of bacterial origin was therefore considered to be of interest.

This paper describes the evaluation of the molecular dimensions of the enzyme and the effects of pH and different enzyme inhibitors on its activity. The influence of intravenously injected enzyme on the kininase activity in rat blood has also been examined.

## METHODS

*Enzyme preparation.* The kininase from *Pseudomonas aeruginosa* was purified according to the procedures described in a previous paper (Rugstad, 1967).

*Determination of kininase activity* was carried out by incubating synthetic bradykinin (BRS 640, Sandoz, Basle, Switzerland) with the enzyme solution. Aliquots of the mixture were taken out at intervals and tested on the rat uterus preparation for remaining bradykinin activity (Rugstad, 1966).

*One unit of kininase* is defined as the amount of enzyme which will destroy 75% or more of 500 ng bradykinin in 11 min under standardized conditions (Rugstad, 1966).

*The effect of pH* on the kininase activity was estimated using the borate-citrate-phosphate buffer described by Teorell & Stenhagen (1938). This buffer may be used over a pH range from 2 to 12. The desired pH was obtained by adding a suitable amount of HCl ; the concentrations of the other ions were kept constant. The pH was measured with a pH meter, type PHM 28 (Radiometer, Copenhagen).

A stock solution of enzyme, which had been dialysed against distilled water, was used for these experiments. Each test solution was prepared by diluting this enzyme preparation more than 1,000 times with the buffer of the desired pH. The final pH in the different test solutions was determined before any assay of kininase activity was carried out. In each experiment the amount of enzyme taken was that which had an activity of one unit of kininase in a Teorell–Stenhagen buffer at pH 7.5. The time needed for inactivation of 75% or more of 500 ng synthetic bradykinin by this amount of enzyme at a particular pH was then measured as described above.

*Standard proteins.* Lyophilized horse myoglobin and 3 times crystallized, salt-free ovalbumin were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A. Crystallized and lyophilized bovine serum albumin and 5 times crystallized ribonuclease-A, type 1A, were obtained from Sigma Chemical Company, St. Louis, Missouri, U.S.A.

*The protein content* of fractions from gel filtration and ultracentrifugation was determined by measuring the optical density at 280 mμ in a Zeiss spectrophotometer (Model PMQ II). The myoglobin content was determined by measuring the optical density at 410 mμ.

*Enzyme inhibitors* The reagents used were of analytical quality. The metal salts used ($CoCl_2$, $FeCl_2$, $ZnCl_2$, $MgSO_4$, NaF and NaCN), and thioglycollic acid were obtained from E. Merck AG, Darmstadt, W-Germany. Disodium edetate, $\alpha\alpha'$-dipyridyl, iodoacetamide and sodium-iodoacetate were obtained from Fluka AG, Buchs, Switzerland. A trypsin inhibitor from ovomucoid, type II-o, l-cysteine-hydrochloride, l-argenine, 1,10 phenanthroline (o-phenanthroline), 2,3 dimercaptopropanol (B.A.L.), N-ethylmaleimide, p-chloromercuribenzene sulphonic acid and p-hydroxymercuri-benzoate were obtained from Sigma Chemical Company. Trasylol (a polypeptide extract of bovine origin) was obtained from Bayer, Leverkusen, W-Germany: this material has an inhibitory effect on many proteolytic enzymes, including several kallikreins. Soybean trypsin inhibitor was obtained from Nutritional Biochemicals Company. Diisopropylfluorophosphate (DFP) was obtained from the Norwegian Defence Research Establishment and was used within 3 hr.

The metal salts and the metal binding reagents were dissolved in 0.05 M Tris-HCl buffer at pH 7.5. The other inhibitors were dissolved in 0.02 M Na-phosphate buffer at pH 7.5. Each stock solution was prepared with a concentration of inhibitor ten times that desired in the final test incubate, and its pH was adjusted to 7.5. The stock solutions of enzyme inhibitors were used within 3 hr. The solutions of sodium iodoacetate, iodoacetate and N-ethylmaleimide, however, were used immediately after they had been prepared.

*Tests for enzyme inhibition.* 0.1 ml. of the stock solution of an inhibitor was incubated for 10 min at 37° C with 0.8 ml. of a solution of the enzyme containing 1.25 u. of activity/ml., made up in the same buffer solution. After this preincubation, 0.1 ml. saline containing 500 ng synthetic bradykinin was added, and the kininase activity was evaluated in the usual way. The kininase activity of the enzyme solution to which the inhibitor had been added was compared with that of the same enzyme solution equally diluted with the buffer only, which should contain one u. of activity/ml.

*Methods for evaluation of molecular dimensions*

*A. Gel filtration.* Sephadex-G-100 (lot No. 7120, obtained from Pharmacia, Uppsala, Sweden) was allowed to swell in 0.02 M Na-phosphate buffer of pH 7.5 for 2 weeks. It was packed in a Sephadex laboratory column, 2½ cm×100 cm, with a gel bed height of 69 cm, according to the method of Flodin (1962a). The sample volume applied to the column was 5 ml. and the flow rate was kept at 12–15 ml./hr. Unless otherwise stated, a 0.02 M Na-phosphate buffer of pH 7.5 was used in the gel filtration and also as a solvent for the enzyme and the standard proteins. Fractions of 5 ml. each were collected with an automatic fraction collector. The gel filtration was carried out at 5° C. The fraction with the highest enzyme activity or, when a standard protein was used, the fraction with the highest absorbency at 280 mμ, was used for the calculation of elution volumes ($V_e$). Blue Dextran 2000 (Pharmacia), F (the fraction with the highest absorbency at 280 mμ), was used for the estimation of the void volume ($V_o$). For the gel filtration column used, this was calculated to be 105 ml., and the internal volume ($V_i$) (Flodin, 1962a) to be 200 ml. The types and concentrations of the standard proteins used in the gel filtration procedures are given in Table 1.

*B. Ultracentrifugation.* Ultracentrifugation was performed with an International Model B-35 preparative ultracentrifuge (International Equipment Comp., Needham Heights, Mass., U.S.A.) with a swinging bucket rotor, Type SB-269. Centrifugation was performed through a medium containing a linearly increasing sucrose gradient (5 to 20%) in 0.02 M Na-phosphate buffer at pH 7.5, as described by Martin & Ames (1961). The medium with the sucrose gradient was prepared in polypropylene tubes in portions of 12.5 ml. and stored for 18 hr at 5° C. The tubes were then kept at 20° C for 1 hr and 0.2 ml. of the protein solution was layered on top of the gradient. The distance

from rotor center to gradient meniscus was 64.5 mm, to the middle of the protein layer 63.5 mm and to the bottom of the tube 151 mm. Centrifugation was carried out with six tubes at a time at 35,000 revs/min for 24 hr; the temperature was 20° C. The same standard protein solution or enzyme solution was always applied to two tubes, and the mean value from these was used in the calculations. Samples were taken from the tubes by making a hole in the bottom with a fractionator similar to that described by Martin & Ames (1961). Fractions of 8 drops each were collected, using a fraction collector with a drop counter. The distance from the middle level of the applied protein layer to the middle layer of the fraction with the highest concentration of the protein was taken as the distance travelled by the protein.

In one set of experiments 0.2 M Tris HCl buffer with 2 M urea added was used instead of the phosphate buffer for making the sucrose gradients and also as a solvent for the enzyme and the standard proteins.

*Kininase activity in plasma after intravenous injection of enzyme*

Rats weighing 200–250 g were anaesthetized by intraperitoneal injections of 0.30 mg/kg pentobarbitone (*Nembutal*®, Abbott Laboratories Ltd.). The animals were tracheotomized and a plastic catheter was inserted in the jugular vein and another in the carotid artery. Heparin, 10,000 i.u./kg, was then given intravenously. One ml. of a solution in 0.9% NaCl of purified enzyme with a kininase activity of 3,000 u./ml. was injected intravenously into each animal. Portions of 0.5 ml. of blood were taken from the carotid artery before the injection of enzyme and again, 5, 10, 20, 60 and 120 min after the injection. Each blood sample was mixed with 9 vol. buffered saline and the mixture centrifuged for 15 min. at 1,000 × *g*. The supernatant was assayed for kininase activity. Only results obtained from animals which survived anaesthesia for at least 3 hr were used.

In one set of experiments anaesthetized rats weighing from 200 to 250 g were injected intravenously with 1 ml. enzyme solution containing 3,000 u./ml. and the effect of intravenously injected synthetic bradykinin on the arterial blood pressure was measured before and after the injection of enzyme. The blood pressure was recorded from the carotid artery with a Statham pressure transducer (P23 De) connected to a Sanborn Model 320 Dual channel DC amplifier-recorder equipped with a Sanborn preamplifier.

## RESULTS

### *Evaluation of molecular dimensions of the kininase*

*A. Gel filtration.* Solutions of the purified enzyme in 0.02 M Na-phosphate buffer at pH 7.5 were submitted to gel filtration as described in the section on methods; the concentrations of enzyme ranged from about 50 to about 5,000 u./ml. Table 1 shows their elution volumes and those of standard proteins. The elution volume was independent of the concentration, both with the enzyme and with the standard proteins, and the value for the enzyme was the same as that reported for the crude supernatant from a culture of *Ps. aer.* (Rugstad, 1967). In Fig. 1 the elution volumes are plotted against log molecular weight, and with the standard proteins a straight line is obtained, as observed by Andrews (1965). From this graph the elution volume of the enzyme corresponds to a molecular weight of about 19,000. Gel filtration of the enzyme and standard proteins was also carried out using 0.2 M Tris-TCl buffer with 2 M urea instead of the phosphate buffer, and similar results were obtained.

*B. Determination of the sedimentation constant of the enzyme.* Ultracentrifugation was carried out as described in the section on methods, using standard proteins with known sedimentation constants as markers (Martin & Ames, 1961). If it is assumed that

TABLE 1

ELUTION VOLUMES OF KININASE AND STANDARD PROTEINS ON A SEPHADEX G-100 COLUMN

| | Mol. w. | Log. mol. w. | Concentration (mg/ml.) | In phosphate buffer Ve | In phosphate buffer Ve/Vo | In Tris-HCl buffer Ve/Vo |
|---|---|---|---|---|---|---|
| | approx. | | | | | |
| Blue Dextran 2000 | 2,000,000 | | 0·5–2 | 105 | 1 | 1 |
| Bovine serum albumin | 67,000[1] | 4·8261 | 1–2 | 130 | 1·24 | |
| Ovalbumin | 47,000[2] | 4·6532 | 1–2 | 155 | 1·48 | 1·58 |
| | | | approx. | | | |
| *Pseudomonas aer.* kininase | 19,280 | 4·285 | 0·002–0·2 | 225 | 2·14 | 2·03 |
| Myoglobin | 17,000- | 4·2304 | 0·5–2 | 235 | 2·24 | 2·21 |
| Ribonuclease | 13,700- | 4·1367 | 1·5–2 | 250 | 2·38 | |

Each protein solution was applied separately to the Sephadex G-100 column (2·5 × 69 cm). V = void volume, calculated from the results with Blue Dextran 2000. Ve = elution volume. For the mol. w. for the standard proteins, see Gutfreund (1944); Kendrew (1949); Hirs, Moore and Stein (1956); Phelps & Putnam (1960). The mol. w. of the kininase is calculated from the log mol. w. obtained in the calibration curve shown in Fig. 1.

the partial specific volume of the enzyme is 0.725 $cm^3/g$, the sedimentation constant $S_{20,w}$ can be estimated from the ratio

$$\frac{\text{distance travelled by unknown protein}}{\text{distance travelled by standard protein}} = \frac{S_{20,w} \text{ of unknown protein}}{S_{20,w} \text{ of standard protein}}$$

The results from the ultracentrifugations are shown in Table 2. The sedimentation constant of the kininase was calculated to be between 3.5 and 3.7, which is a little higher

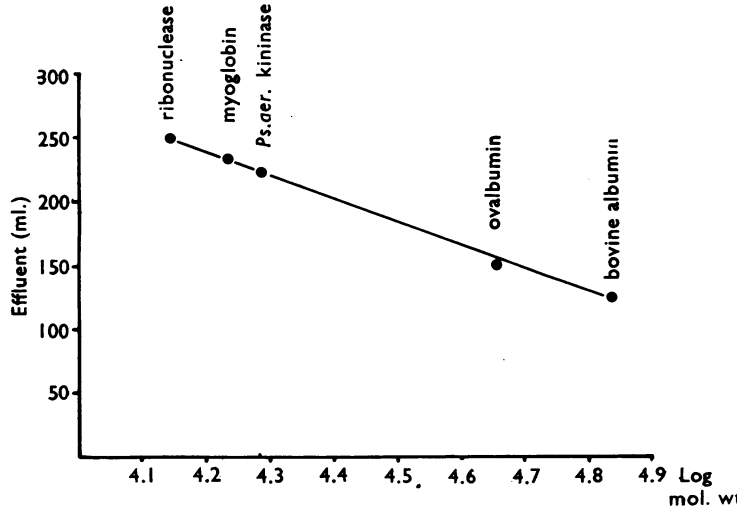


Fig. 1. Calibration curve for a Sephadex G-100 column (2.5×69 cm) used for determination of molecular weights according to Andrews (1965). The elution volumes of different standard proteins are plotted against their log mol. wt. (see Table 1). The elution volume of the kininase from *Ps. aer.* has been used to estimate its molecular weight.

TABLE 2

ESTIMATION OF SEDIMENTATION CONSTANT OF THE KININASE FROM *PS. AER.* BY ULTRACENTRIFUGATION THROUGH A SUCROSE GRADIENT

| Reference protein | Calculated sedimentation constant of the kininase ($S_{20,w}$) | |
|---|---|---|
| | In 0·02M Na-phosphate buffer pH 7·5 | In 0·2M Tris-HCl buffer pH 7·5 with 2M urea |
| Ribonuclease ($S_{20,w}=1·87$[1]) | 3·5 | |
| Ovalbumin ($S_{20,w}=3·42$[2]) | 3·7 | 3·5 |
| Bovine serum albumin ($S_{20,w}=4·41$[3]) | 3·5 | 3·5 |

The sedimentation constant of the kininase was estimated by ultracentrifugation at 20° C through a sucrose gradient (see Methods) using proteins with known $S_{20,w}$ values as references. The concentration of the standard proteins was 2 mg/ml. and of the kininase 0·15 mg/ml. For the sedimentation constants of the reference proteins shown in parentheses see Ginsburg, Appel & Schachman (1956); Creeth & Winzor (1962); Phelps & Putnam (1960).

than that of ovalbumin. Ultracentrifugation was also carried out using a 0.2 M Tris-HCl buffer of pH 7.5 containing 2 M urea instead of the Na-phosphate buffer in the sucrose gradient. With this buffer, too, the distance travelled by the enzyme was almost the same as the distance travelled by ovalbumin (see Table 2).

*The influence of pH on the activity of the kininase* was determined in the buffer described by Teorell & Stenhagen (1938). Because the content of HCl varies in this buffer at different pH's whereas the content of other ions is kept constant, a preliminary experiment was performed to observe the effects of a high concentration of $Cl^-$-ions. The enzyme was incubated with NaCl in a concentration giving twice the $Cl^-$-ion concentration in the Teorell-Stenhagen buffer at pH 2. This did not apparently inhibit the destruc-

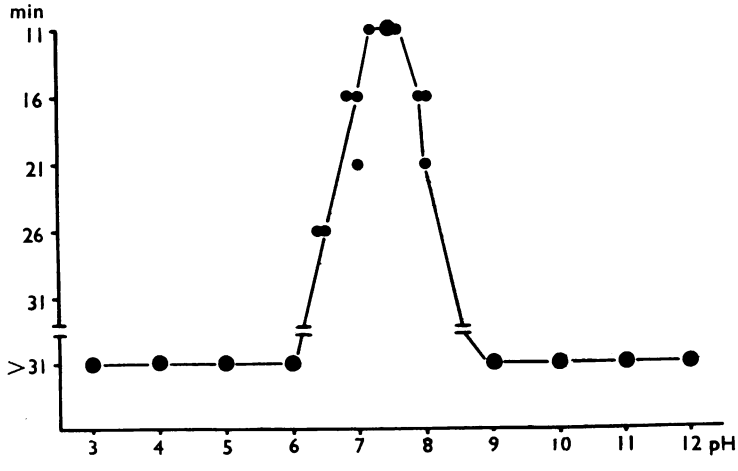


Fig. 2. The influence of pH on the inactivation of bradykinin by the kininase from *Ps. aer.* 0.1 ml. of a solution containing synthetic bradykinin (5 $\mu$g/ml.) was incubated with a standard amount of kininase in borate-citrate-phosphate buffer solutions of different pH's, and the time needed to inactivate 75% or more of the bradykinin was determined (see Methods). The small dots indicate results from single tests at the pH level indicated, large dots indicate the results of two or more tests at the same pH.

tion of bradykinin by the enzyme, consequently any difference in the rate of inactivation of bradykinin over the pH range from 3 to 12 with this buffer can be attributed to the difference in hydrogen concentration. The effects of pH on kininase activity are shown in Fig. 2. The enzyme has a pH optimum of about 7.5, and its activity diminishes rapidly above or below this value.

*The effect of enzyme inhibitors on the activity of the kininase* is shown in Table 3. The metal binding reagents 2,3-dimercaptopropanol (BAL) and 1,10-phenantroline were potent inhibitors, whereas other metal binding substances, such as disodium edetate,

TABLE 3

EFFECT OF VARIOUS ENZYME INHIBITORS ON THE ACTIVITY OF KININASE FROM *PS. AER.*

Kininase activity was estimated by mixing 1 u. kininase with 500 ng synthetic bradykinin and recording the time for its break-down (see Methods). A pronounced inhibition is marked ++. This implies a prolongation of the break-down period of the certain amount of bradykinin from 11 min to 31 min or more. A less pronounced inhibition is marked + and no inhibition is indicated by 0

| Inhibitors | Concentration | Degree of inhibition |
|---|---|---|
| $CoCl_2$ | $3\times10^{-3}$M | + |
| $ZnCl_2$ | $1{\cdot}5\times10^{-3}$M | ++ |
| $MgSO_4$ | $3\times10^{-3}$M | 0 |
| $FeCl_2$ | $3\times10^{-3}$M | 0 |
| NaCN | $3\times10^{-2}$M | 0 |
| NaF | $2\times10^{-2}$M | 0 |
| Disodium edetate | $1{\cdot}5\times10^{-2}$M | 0 |
| Disodium edetate | $1{\cdot}5\times10^{-4}$M | 0 |
| $\alpha\alpha'$-dipyridyl | $3\times10^{-2}$M | 0 |
| 1,10 Phenanthroline | $1{\cdot}5\times10^{-4}$M | + |
| 1,10 Phenanthroline | $3\times10^{-3}$M | ++ |
| 2,3-dimercaptopropanol (BAL) | $1\times10^{-3}$M | ++ |
| Na-thioglycollate | $1\times10^{-2}$M | 0 |
| Cysteine | $3\times10^{-3}$M | (+)0 |
| Iodoacetamide | $3\times10^{-3}$M | 0 |
| Na-iodoacetic acid | $1\times10^{-2}$M | 0 |
| N-ethylmaleimide | $1\times10^{-3}$M | 0 |
| p-chloromercuribenzene-sulphonic acid | $3\times10^{-4}$M | + |
| p-chloromercuribenzene-sulphonic acid | $3\times10^{-3}$M | ++ |
| p-hydroxymercuribenzoate | $3\times10^{-4}$M | + |
| Trasylol | 150 u./ml. | 0 |
| Ovomucoid trypsin inhibitor | 0·6 mg/ml. | 0 |
| Soybean trypsin inhibitor | 0·6 mg/ml. | 0 |
| Diisopropyl fluorophosphate | $1\times10^{-2}$M | 0 |

sodium cyanide, sodium fluoride and $\alpha\alpha'$-dipyridyl had no detectable influence on the enzymatic activity. N-Ethylmaleimide, iodoacetamide and sodium iodoacetate, which inhibit enzymes containing sulphydryl groups, had no detectable inhibitory effect, whereas p-chloromercuribenzene sulphonic acid showed weak effects in a concentration of $3\times10^{-4}$ M but marked inhibition at $3\times10^{-3}$ M.

*Kininase activity in plasma after intravenous injection of enzyme.* Fig. 3 shows the levels of kininase activity after 3,000 u. kininase had been injected intravenously into anaesthetized rats. Ten minutes after the injection of the enzyme the total kininase

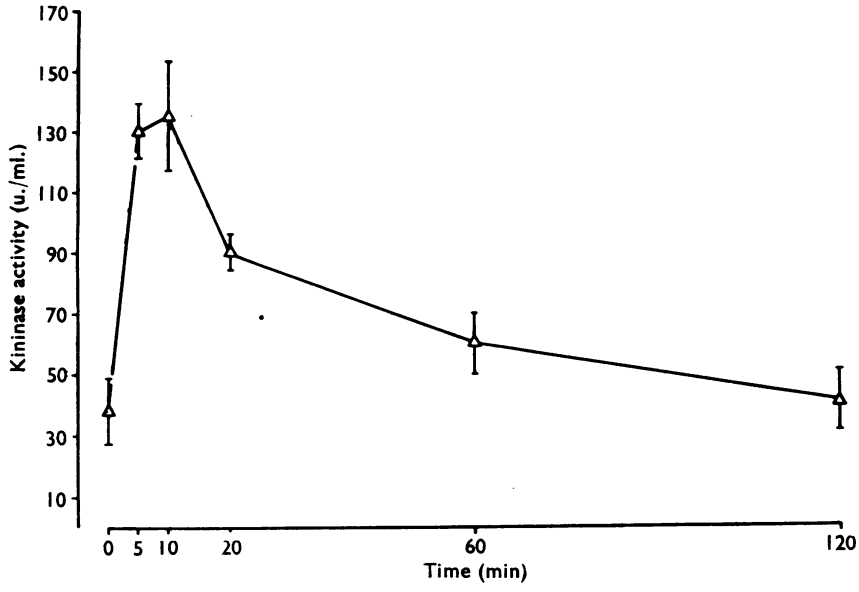


Fig. 3. The effect of intravenous injections in rats of 3,000 u. of kininase from *Ps. aer.* on the total plasma kininase activity. The arithmetic mean, ± the standard deviation of the mean of values from 5 rats is plotted against the time. At zero time the initial values of kininase in the rat plasmas were determined and then 1 ml. of a solution containing the kininase was given intravenously. For kininase determination and the definition of one unit of kininase, see Methods.

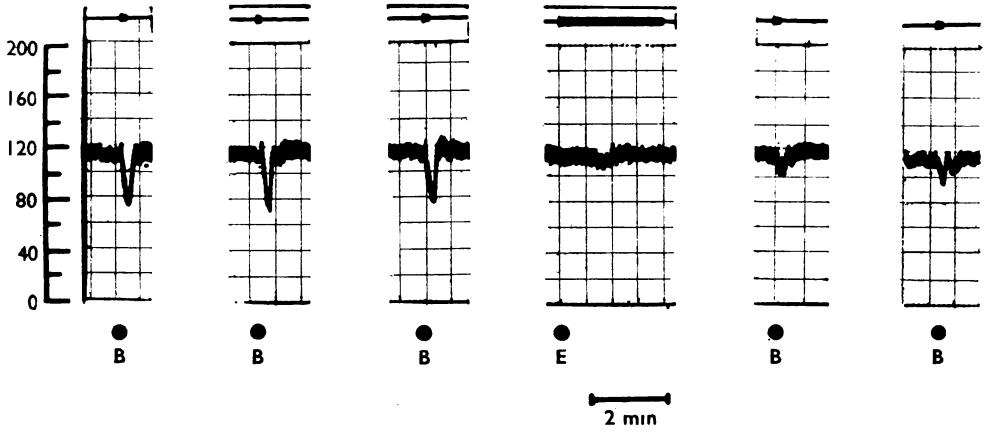


Fig. 4. The effect of intravenously injected kininase from *Ps. aer.* on the blood pressure responses to injected synthetic bradykinin. Rat, 200 g, anaesthetized with pentobarbitone. At B bradykinin, 1 μg/kg, was injected into the femoral vein. At E 1 ml. of a solution, containing 3,000 u. of kininase from *Ps. aer.*, was injected into the jugular vein. The interval between injections was 5 min.

activity in the plasma was three to five times the activity before the injection and the activity remained considerably higher than normal for at least 60 min. The half-life of the increased kininase in the plasma was about 20 min.

The effect of synthetic bradykinin on the arterial blood pressure of anaesthetized rats was tested before and after the intravenous injection of kininase. Bradykinin in doses of from 0.5 $\mu$g to 2 $\mu$g/kg body weight was injected either into the jugular vein or into the femoral vein. The fall in blood pressure obtained by a selected dose of bradykinin injected into the femoral vein was significantly reduced after the intravenous administration of 3,000 u. of purified enzyme (Fig. 4). This effect could be seen approximately half an hour after the injection of enzyme. The blood pressure response to doses of bradykinin injected into the jugular vein, however, was much less reduced after administration of this amount of enzyme.

## DISCUSSION

*The molecular weight* of the kinin-inactivating enzyme from *Ps. aer.* was found to be about 19,000 when evaluated from the results of gel filtration. This is not in agreement with the value of the sedimentation coefficient ($S_{20,w}$) which was found to be between 3.5 and 3.7. Such a coefficient is slightly higher than that of ovalbumin, which has a molecular weight of 47,000. One possible explanation for this discrepancy may be that the enzyme has been adsorbed on to the Sephadex gel during filtration, and that its molecular weight is really about the same as that of ovalbumin. It is known that adsorption of proteins to Sephadex gels can take place. Whitaker (1963) found that lysozyme was eluted from a Sephadex G-100 column much later than expected. It behaved there as if it had a molecular weight of 7,700, which is only one half its molecular weight as determined by other methods. Gelotte (1960) has discussed the adsorption properties of the Sephadex gel material and Flodin (1962b) has described adsorption and other side-effects occurring in Sephadex gel filtration columns.

For the calculation of the sedimentation constant a partial specific volume of 0.725 $cm^3/g$ protein has been assumed. The partial specific volume of most proteins lies between 0.700 and 0.750 $cm^3/g$ (Edsall, 1953), so there may be a small error in the estimation of $S_{20,w}$ for the kininase. This will only lead to a small error in the estimate of the molecular weight, however, and could not account for the difference from the result obtained by gel filtration.

Kinin-destroying activity is present in blood plasma, in lymph and in red blood cells (review by Trautschold & Rüdel, 1963), and also in various other tissues (Amundsen & Nustad, 1965). Studies on kininases from these sources have been carried out with rather crude preparations such as haemolysates, tissue homogenates or various plasma fractions. The effects of different enzyme inhibitors on the activity of the various kininases has been the subject of several studies. Erdös & Sloane (1962) found that the kininase activity in human plasma fraction IV-1 was inhibited by phenanthroline, disodium-edetate and some other substances but not by DFP (Diisopropylfluorophosphate). A pronounced inhibitory effect of thioglycolic acid on plasma kininase was described by Werle, Hochstrasser, Trautschold & Leysath (1964). The kininase activity from red blood corpuscles has been found by Erdös, Renfrew, Sloane & Wohler (1963)

to be markedly inhibited by phenanthroline, $\alpha\alpha'$-dipyridyl and p-chloromercuribenzene sulphonic acid, but not by disodium edetate. Amundsen & Nustad (1964) confirmed the results of Erdös & Sloane (1962) and Erdös *et al.* (1963) as regards the effect of various inhibitors on the kininases in plasma and from erythrocytes. As judged from the present results with enzyme inhibitors, the kininase from *Ps. aer.* differs in its activity from both the plasma kininase and the kininase in red blood cells. It should be borne in mind, however, that the two latter kininases have been studied in rather crude extracts only.

Two purified well-characterized enzymes inactivate bradykinin—namely, carboxypeptidase B and chymotrypsin (Erdös *et al.*, 1963). Carboxypeptidase B has been purified and characterized by Folk, Piez, Carroll & Gladner (1960). They found that the enzyme had a sedimentation constant ($S_{20,w}$) of 3.23 and a molecular weight of 34,300. With hippuryl-l-arginine as substrate, the enzyme was completely inhibited by $\alpha\alpha'$-dipyridyl in a concentraton of $3.3 \times 10^{-4}$ M. The purification and characterization of chymotrypsin has been reviewed by Desnuelle (1960), who states that the molecular weight lies between 21,500 and 27,000. Both the proteinase activity (substrate: haemoglobin) and the esterase activity (substrate: l-tyrosine ethyl ester) of chymotrypsin is completely inhibited by diisopropylfluorophosphate (DFP; Jansen, Nutting, Jang & Balls, 1949).

The kininase from *Ps. aer.* seems to differ from carboxypeptidase B and chymotrypsin both as regards molecular weight and as regards the influence of various enzyme inhibitors. The comparisons are, however, not quite satisfactory as regards enzyme inhibition since bradykinin has not been used as substrate in the studies with chymotrypsin and carboxypeptidase B.

The kininase from *Ps. aer.* was not inhibited by N-ethylmaleimide, iodoacetamide or Na-iodoacetate. It does not, therefore, appear that sulphydryl groups are essential for the activity of this enzyme. The inhibitory effect of high concentrations of p-chloromercuribenzene sulphonic acid and p-hydroxymercuri-benzoate is probably due to some action other than interference with sulphydryl groups.

The kininase from *Ps. aer.* is remarkably stable in blood from humans and rats. No loss of activity could be detected after *in vitro* incubation with blood at 37° C for 24 hr (Rugstad, 1967). After intravenous injection of purified enzyme in rats the increase in plasma kininase activity declined with a half-life of about 20 min. In cats Erdös & Yang (1966) found a half-life in plasma of about 30 min for intravenously injected pancreatic carboxypeptidase B, whereas the same enzyme has been found to have a half-life of about 17 min in rabbits (Erdös, Wohler & Levine, 1963). The half-life of a particular enzyme can thus differ from one species to another. Amelung (1960) found that enzymes with the same molecular weights had very different half-lives in rabbits, their eliminations apparently being influenced by several processes. With the good *in vitro* stability in blood of the kininase from *Ps. aer.* it seems likely that the decline in the effect of intravenously injected enzyme should be caused by passage of enzyme molecules out through the capillary walls with disappearance from the circulation.

Intravenously given carboxypeptidase B has been shown to abolish or very much diminish the hypotensive response to bradykinin in guinea-pigs, rabbits and cats, whereas chymotrypsin did not have such an effect *in vivo* (Erdös *et al.*, 1963). The kininase from *Ps. aer.* can reduce the hypotensive effect of bradykinin when given intravenously. This

reduction is more clearly seen when the bradykinin is injected through the femoral vein than when injected through the jugular vein. This is probably because the time needed for the bradykinin to reach the effector site(s) is longer when the injection is made into the femoral vein, so bradykinin will be more liable to inactivation by the injected enzyme.

The results obtained with this kininase of bacterial origin suggest that it may diffuse fairly easily from a focus of infection without being destroyed or inactivated. It could thus reduce the amount of free kinins in the vicinity of an infected area. Possibly the ability of the microbe to produce a stable kininase is related to its pathogenicity.

SUMMARY

1. A purified kinin-inactivating enzyme which is produced by *Pseudomonas aeruginosa* has been further examined.

2. The results of gel filtration on Sephadex G-100 indicated that the molecular weight of the kininase was about 19,000. The sedimentation constant ($S_{20,w}$) as determined by sucrose gradient centrifugation was, however, found to be between 3.5 and 3.7. The discrepancy between these findings is discussed. It seems most likely that the enzyme has a molecular weight of about 50,000 corresponding to a sedimentation coefficient of about 35.

3. The pH optimum of the enzyme with synthetic bradykinin (Sandoz) as substrate is 7.5 and its activity decreases rapidly above or below this pH.

4. The enzyme is inhibited by o-phenanthroline and 2,3-dimercaptopropanol (BAL), but not by disodium edetate, diisopropylfluorophosphate or $\alpha\alpha'$-dipyridyl. These findings show that the kininase is probably different in its activity from the kininases hitherto characterized.

5. When 0.15 mg of the enzyme is injected intravenously into rats, the kininase activity of the plasma is increased 3–5 fold and the hypotensive effect of injected synthetic bradykinin is antagonized. The half-life of the injected enzyme is about 20 min.

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