α -1 Anti-Trypsin, an Inhibitor of Renin

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(Received 1 December 1975)

A naturally occurring competitive inhibitor of pig kidney renin has been identified in human plasma. The inhibitor was shown to be α -1 anti-trypsin and the effect *in vitro* on the renin activity was examined. The slope in the Hill plot is compatible with the assumption of a one-site competitive inhibition. Other proteinase inhibitors such as α -2 macroglobulin and C1 inactivator, however, have no inhibitory effect on the renin-angiotensinogen reaction.

Renin (EC 3.4.4.15) is a proteinase of renal origin that splits a Leu-Leu bond in an α -2 globulin, angiotensinogen, to yield a decapeptide, angiotensin I. Angiotensin I is subsequently converted enzymically into angiotensin II, an octapeptide with several major biological functions.

Although renin is only a trace enzyme of tissue and plasma, it is surprising that no plasma inhibitor, except the angiotensin-inactivating peptidases, has yet been found for this very important renin-angiotensin system, as long-acting vasopressor activity is provided by the angiotensin II resulting from the reaction sequence.

The renin-inhibitory role of a lysophospholipid of renal origin (Smeby *et al.*, 1967) is now questioned by the results of the experiments of Osmond *et al.* (1973). Haemoglobin can exert an inhibitory effect (Workman *et al.*, 1974), but it is present in plasma at far too low a concentration to account for any significant portion of plasma's inhibitory effect.

In the present paper we report that α -1 anti-trypsin is a potent inhibitor of renin, whereas α -2 macroglobulin, soya-bean trypsin inhibitor and C1 inactivator have practically no inhibitory effect on this enzyme.

Experimental

Purified pig renin preparation, prepared by the procedure of Haas *et al.* (1953), was purchased from Nutritional Biochemical Corp., Cleveland, OH, U.S.A., and its activity was 17 Goldblatt units/mg. It was processed according to step 8 of the procedure of Haas *et al.* (1953) to remove angiotensinase activity. Purified pig plasma substrate was purchased from Miles Laboratories, Maidenhead, Berks., U.K. This contained no detectable renin, converting enzyme or angiotensinases. α -1 Antitrypsin and α -2 macro-

globulin were obtained from the American National Red Cross, Blood Research Laboratory, Bethesda, MD, U.S.A. Soya-bean trypsin inhibitor was purchased from Worthington Biochemical Corp., Freehold, NJ, U.S.A., and C1 inactivator was a gift from the Central Laboratory of the Netherlands Red Cross, Amsterdam, The Netherlands.

The renin assay system that we normally used (Eid *et al.*, 1976) in these studies combines an incubation of pig kidney renin, prepared by the procedure of Haas *et al.* (1965), for 8 min at 37°C at pH 7.5 with 0.48 nmol of purified pig plasma renin substrate/ml, free of converting enzyme and angiotensinases. The angiotensin I liberated was measured by a modification of the radioimmunoassay technique of Haber *et al.* (1969) and calibrated with angiotensin I obtained from the National Institute of Biological Standards and Control, London, U.K.

Results and Discussion

The plots of the uninhibited and inhibited velocities of angiotensin I formation as a function of the concentration of substrate and the concentration of α -1 anti-trypsin, according to the methods of Lineweaver & Burk (1934) (Fig. 1), Eadie (1942) and Dixon (1953), all indicate a competitive-inhibition mechanism. Using the same equations, we derived the apparent Michaelis-Menten constant (0.17 μ M), the uninhibited rate at maximum substrate concentration (1 nmol/min) and the inhibitor constant K_i (0.3 μ M).

When the kinetic data are plotted by the logarithmic method of Hill (Loftfield & Eigner, 1969) we find a slope of 1 (Fig. 2), indicating that one molecule of α -1 anti-trypsin (in the concentration range studied) is reacting with one molecule of enzyme.

Fig. 1. Lineweaver-Burk plots of the inhibitory effects of two different concentrations of human α -1 anti-trypsin on renin

The concentrations of α -1 anti-trypsin used were: ●, none; ▲, $0.25 \mu\text{M}$; ■, $0.625 \mu\text{M}$. Other incubation conditions were: renin concentration, 4.3×10^{-3} Goldblatt units; substrate concentration, $0.48 \mu\text{M}$; temperature of incubation, 37°C .

The competitive inhibition of renin, being a leucyl-leucyl endopeptidase, is consistent with the hypothesis of Cohen (1973) that α -1 anti-trypsin contains not only a positively charged amino acid but also a leucine residue in its active/inhibitor sites.

These investigations of the inhibition of renin may explain not only the results of the experiments of Winch *et al.* (1972), which attribute to plasma a considerable renin-inhibitory effect, but also the trypsin-induced increase in plasma renin activity described by Cooper *et al.* (1974).

The inhibition of renin by a plasma proteinase inhibitor also evokes an analogy with the kallikrein-kinin system, where the production of potent vasodilating hypotensive peptides is controlled at three different levels: (a) Hageman factor by C1 inactivator, (b) kallikrein by α -2 macroglobulin, C1 inactivator and anti-thrombin III, and finally (c) hydrolysis of the kinins by inactivating peptidases.

The biological role of plasma inhibitors is determined not only by their affinity for certain

Fig. 2. Inhibition of renin by α -1 anti-trypsin

Uninhibited (v) and inhibited velocities (v_i) of angiotensin I production are plotted by the method of Hill. The data fall on a straight line with a slope value of 1, suggesting that one molecule of inhibitor is involved in the interaction with the active centre of the enzyme. The concentrations of α -1 anti-trypsin ranged from 1.25 to $25 \mu\text{M}$.

proteinases and their molar concentration in plasma, but largely by their ability to diffuse into the extravascular space and their capacity to become enriched in certain biological fluids. About 90% of labelled α -1 anti-trypsin has been found to be shifted to the extravascular space during a 48 h observation time (Kueppers & Fallat, 1969).

The high plasma molar concentration of α -1 anti-trypsin, which is also an inhibitor of granulocyte collagenases and elastases (preventing tissue proteolysis) and of activated factor XI, thrombin and plasmin, underlines the general importance of this anti-proteinase.

As suggested by Pannell *et al.* (1974), α -1 anti-trypsin could be more accurately described as α -1 proteinase inhibitor.

References

- Cohen, B. (1973) *J. Biol. Chem.* **248**, 7055-7059
- Cooper, R., Osmond, D., Scaiff, K. & Ross, L. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 584
- Dixon, M. (1953) *Biochem. J.* **55**, 170-177
- Eadie, G. (1942) *J. Biol. Chem.* **146**, 85-93
- Eid, M., Scharpé, S. & Lauwers, A. (1976) *Protides Biol. Fluids Proc. Colloq.* **23**, in the press
- Haas, E., Lamfrom, H. & Goldblatt, H. (1953) *Arch. Biochem. Biophys.* **42**, 63-78
- Haas, E., Goldblatt, H. & Gipson, E. C. (1965) *Arch. Biochem. Biophys.* **110**, 534-543

- Haber, E., Koerner, T., Page, L. B., Kliman, B. & Purnode, A. (1969) *J. Clin. Endocrinol. Metab.* **29**, 1349–1355
- Kueppers, F. & Fallat, R. (1969) *Clin. Chim. Acta* **24**, 401–403
- Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658–666
- Loftfield, R. & Eigner, E. (1969) *Science* **164**, 305–308
- Osmond, D., McFadzean, P. & Ross, L. (1973) *Proc. Soc. Exp. Biol. Med.* **144**, 969–973
- Pannell, R., Johnson, D. & Travis, J. (1974) *Biochemistry* **13**, 5439–5445
- Smeby, R., Sen, S. & Bumpus, F. (1967) *Circ. Res.* **20–21**, Suppl. 2, 129–133
- Winch, M., Versey, J., Noble, A. & Munday, K. (1972) *Experientia* **28**, 1394–1395
- Workman, R., McKown, M. & Gregerman, R. (1974) *Biochemistry* **13**, 3029–3035