Inhibition of Angiotensin – Converting Enzyme by a Synthetic Peptide Fragment of Glyceraldehyde-3-phosphate Dehydrogenase

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A novel inhibitor of angiotensin – converting enzyme (ACE) identical to a sequence part of human muscle glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chemically synthesized. Amino acid sequence was as follows Pro – Glu – Asn – Ile – Lys – Trp – Gly – Asp. This peptide inhibited rabbit lung ACE with a K_i value of 1.6 μM . The inhibitor of ACE acts in a non-competitive manner. The amino acid sequence of the new inhibitor was compared of GAPDH from other sources.

Introduction

The renin – angiotensin system is important in the regulation of blood pressure and angiotensin – converting enzyme (ACE; peptidyl dipeptide hydrolase, EC 3.4.15.1). Inhibitors have been of great value for their hypotensive effect through this system. Competitive inhibitors of ACE were first obtained from venom of snakes such as *Bothrops jararaca* (Cheung *et al.*, 1980) and *Agkistrodon halys blomhoffi* (Kato and Suzuki, 1971). These inhibitors have many proline residues. Another inhibitor of ACE has been isolated from an acid extract of tuna (*Neothunnus macropterus*) muscle (Kohama *et al.*, 1989). This peptide was found to be a non-competitive inhibitor different from many natural peptide inhibitors (Kohama *et al.*, 1994). The amino acid sequence of tuna ACE inhibitor was similar as though not identical, to segments within the vertebrate GAPDH family (Kohama *et al.*, 1989).

Thus the attention of many laboratories were focused on a family of ACE inhibitory peptides excised from vertebrate and prokaryote GAPDH (glyceraldehyde-3-phosphate dehydrogenase, EC 1.2.1.12) by acid-limited proteolysis (Kohama *et al.*, 1989).

In this paper, the inhibitory effect of a synthetic peptide, identical to the peptide 79–86 of the human muscle GAPDH was studied. The complete amino acid sequence of this enzyme was estab-

lished earlier in our laboratory (Nowak *et al.*, 1989). The structure of the human peptide is similar to the active inhibitors of ACE from rat, chicken and tuna muscle glyceraldehyde-3-phosphate dehydrogenase.

Materials and Methods

Materials – Peptidyl dipeptide hydrolase (ACE; EC 3.4.15.1) from rabbit lung, hippuryl-L-histidyl-L-leucine (Hip-His-Leu), L-histidyl-L-leucine (His-Leu) were purchased from Sigma Chemical (Mo., USA).

All others reagents and solvents were obtained from Serva, Germany.

Peptide synthesis – The peptide: Pro-Glu-Asn-Ile-Lys-Trp-Gly-Asp was synthesized by conventional solid phase method using an automated peptide synthesizer (Applied Biosystems Inc., model – 430 A), followed by treatment with hydrogen fluoride to cut off the support resins and to remove all of the protecting groups (Merrifield, 1985). The synthesized peptide was purified by reverse phase high performance liquid chromatography on Develosil ODS-7 column (0.46 $\times$ 25 cm) eluted with a 5–30% acetonitrile in 0.05% HCl (linear gradient). After that the peptide was run on a Asahipak GS-220 column (0.76 $\times$ 25 cm) eluted with 50 mM sodium phosphate buffer, pH 7.0. The results of amino acid analyses and sequence analyses agreed well with expected values.

ACE activity assay – The activity of ACE was assayed by fluorometrically (Cheung *et al.*, 1973) of the rate of formation of histidylleucine from Hip-His-Leu. Incubations were carried out at 37 °C in final volume of 0.25 ml, which contained 100 mM potassium phosphate buffer (pH 8.3), 300 mM NaCl, enzyme (activity 3.4 U/mg) and 2.5 mM Hip-His-Leu. The enzymatic reactions were terminated by addition of 1.45 ml of 0.3 M NaOH and 100 μ l 0.2% o-phthalaldehyde was then added, followed after 10 min. by 0.2 ml of 3 M HCl. The fluorescence was measured with Perkin-Elmer MPF 3L spectrofluorimeter (λ_{ex} =360 nm, λ_{fl} =500 nm).

ACE activity was assayed in the presence or absence of inhibitor and compared to controls containing no inhibitor. In other experiments our inhibitor was preincubated with various concentrations of ZnCl₂ for 5 min. at 37 °C before the reaction was started.

Results

The amino acid sequence of the peptide inhibitor of ACE, obtained synthetically, was as follows: Pro-Glu-Asn-Ile-Lys-Trp-Gly-Asp. This sequence corresponds to the peptide 79–86 from human skeleton muscle GAPDH.

Figure 1 shows the Lineweaver-Burk plot of inhibition of rabbit lung ACE catalyzed hydrolysis of Hip-His-Leu by synthetic peptide. Activity of ACE in control sample (non-inhibited) was 3.4 μ mol · mg protein⁻¹ · min⁻¹. This peptide inhibited the enzyme non-competitively. The K_i value was calculated by Dixon method was 1.6 μ M. I₅₀ value (concentration of inhibitor producing a 50% inhibition of ACE) was 3.7 μ M. With great excess of this inhibitor only about 80% inhibition of ACE was obtained.

It is well known, that EDTA inhibits the activity of ACE by chelating the enzyme bound Zn⁺². This inhibition may be reversed by addition of Zn⁺² – containing solutions to the reaction mixture. Results are shown in Table I. The addition of Zn⁺² – ions reversed completely the inhibition by EDTA, but had only minor influence on the inhibition by the synthetic peptide, diminishing it by 5 and 10%.

When this synthetic peptide (inhibitor) was preincubated without ZnCl₂, its ACE inhibitory activity was about 50%. When 10 or 20 μ M ZnCl₂ was

Fig. 1. Lineweaver-Burk plots of inhibition of rabbit lung ACE by synthetic peptide corresponding to sequence of GAPDH. ACE concentrations was 6 mU for assay, 1 unit of ACE activity is defined as the amount catalyzing the formation of 1 μ mol of hippuric acid from Hip-His-Leu in 1 min. at 37 °C. Inhibitor concentration: ● – 0 μ M, ▲ – 3 μ M, ■ – 6 μ M.

Table I. Effect of ZnCl₂ on inhibition of ACE by synthetic peptide corresponding to sequence GAPDH from human muscle and by EDTA. Synthetic peptide (3 μ M) or EDTA (3 μ M) was preincubated with the indicated concentrations of ZnCl₂ at 37 °C for 5 min before the enzyme reaction was initiated.

Sample	ZnCl ₂ (μ M)	ACE inhibition (%)
Synthetic peptide (12 μ M)	0	80
Synthetic peptide (3 μ M)	6	50
	10	45
	20	41
EDTA (3 μ M)	0	65
	5	6
	15	0

With 12 μ M synthetic peptide an 80% inhibition could be obtained at maximum.

added respective inhibitory activities were 45–41%. The influence of EDTA on ACE – which was added after preincubation with ZnCl₂ is shown in Table I. These data indicate that this synthetic peptide that corresponds to sequence 79–86 from human skeleton muscle GAPDH does interact with Zn⁺² – ions (Krotkiewska and Banaś, 1992).

Discussion

Angiotensin – converting enzyme catalyses the cleavage of the C-terminal dipeptide L-His-L-Leu of the decapeptide angiotensin I, generating the biologically highly active vasopressor angiotensin II.

Since ACE is a dipeptidyl carboxypeptidase, the C-terminal tripeptide fragment of the inhibitory peptides from snake venom is predominant in its competitive binding to the enzyme. The terminal aromatic amino acid is favorable for effective binding of peptide inhibitors to the active site of the enzyme (Kohama *et al.*, 1991). The synthetic peptide shows a non-competitive mode of inhibition. All of the octapeptides in Table II have a dicarboxylic acid Asp as terminal and Gly as penultimate, and the amino acid sequence is different from those of the venom peptide family (Cheung and Cushman, 1973; Cheung *et al.*, 1980). These facts may support a non-competitive inhibition of ACE by these inhibitors. Differences of amino acid sequence evident at the position 80 of GAPDH – derived peptides seem not to influence their inhibitory activity.

ACE contains zinc ions which plays an important role in the active site (Wei *et al.*, 1991). EDTA shows a powerful inhibitory activity by chelating

Table II. Sequence homology of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) peptides.

Position	79	80	81	82	83	84	85	86	Source
AA	P	E	N	I	K	W	G	D	Human (Nowak <i>et al.</i> , 1981)
	P	A	N	I	K	W	G	D	Porcine (Harris and Perham, 1968)
	P	A	N	I	K	W	G	D	Rat (Tso <i>et al.</i> , 1985)
	P	S	N	I	K	W	A	D	Chicken (Domdey <i>et al.</i> , 1983)
	P	T	N	I	K	W	G	D	Tuna (Kohama <i>et al.</i> , 1994)

the ACE active site for zinc. Its main inhibitory mechanism was shown to be a chelating effect, because saturation with exogenous zinc abolishes inhibition by EDTA. However, our synthetic peptide did not show any zinc chelating effect. It seems that its inhibitory mechanism is non-competitive in our assay condition with Hip-His-Leu.

Although our peptide is only a moderately strong inhibitor of ACE, compared to known ACE inhibitors, it is noteworthy that this peptide from human muscle GAPDH inhibits rabbit lung ACE in a non-competitive manner. In pathological states resulting in protein degradation GAPDH could be proteolized. It could be the reason of additional decrease of blood pressure.

- Cheung H. S. and Cushman D. W. (1973), Inhibition of homogeneous angiotensin – converting enzyme of rabbit lung by synthetic venom peptides of *Bothrops jararaca*. *Biochim. Biophys. Acta* **293**, 451–463.
- Cheung H. S., Wang F. L., Ondetti M. A., Sabo E. F. and Cushman D. W. (1980), Binding of peptide substrates and inhibitors of angiotensin – converting enzyme. *J. Biol. Chem.* **255**, 401–407.
- Domdey H., Wiebauer K., Klapthar H. and Arnold H. (1983), Sequence analysis of the cloned mRNA coding for glyceraldehyde-3-phosphate dehydrogenase from chicken heart muscle. *Eur. J. Biochem.* **131**, 129–135.
- Harris J., and Perham R. N. (1968), Glyceraldehyde-3-phosphate dehydrogenase from pig muscle. *Nature* **219**, 1025–1028.
- Kato H. and Suzuki T. (1971), Bradykinin-potentiating peptides from the venom of *Agkistrodon halys blomhoffii*. Isolation of five bradykinin potentiators and the amino acid sequences of two of them, potentiator B and C. *Biochemistry* **10**, 972–980.
- Kohama Y., Kuroda T., Iida K., Ithon S. and Mimura T. (1994), Inhibitory effect of tuna peptide on endothelin production in cultured endothelial cells. *Biol. Pharm. Bull.* **17**, 886–888.
- Kohama Y., Oka H., Kayamori Y., Tsujikawa K., Mimura T., Nagase Y. and Satake M. (1991), Potent synthetic analogues of angiotensin – converting enzyme inhibitor derived from tuna muscle. *Agric. Biol. Chem.* **55**, 2169–2170.
- Kohama Y., Oka H., Matsumoto S., Nakagawa T., Miyamoto T., Mimura T., Nagase Y., Satake M., Takane T. and Fujita T. (1989), Biological properties of angiotensin – converting enzyme inhibitor derived from tuna muscle. *J. Pharmacobiodyn.* **12**, 566–671.
- Kohama Y., Oka H., Yamamoto K., Teramoto T., Okabe M., Mimura T., Nagase Y. and Satake M. (1989), Induction of angiotensin – converting enzyme inhibitor by acid-limited proteolysis of glyceraldehyde-3-phosphate dehydrogenase. *Biochem. Biophys. Res. Commun.* **161**, 456–460.
- Krotkiewska B. and Banaś T. (1992), Interaction of Zn^{+2} and Cu^{+2} ions with glyceraldehyde-3-phosphate dehydrogenase from bovine heart and rabbit muscle. *Int. J. Biochem.* **24**, 1501–1505.
- Merrifield R. B. (1985), Solid-phase synthesis. *Chemica Scripta* **25**, 121–131.
- Nowak K., Wolny M. and Banaś T. (1981), The complete amino acid sequence of human muscle glyceraldehyde-3-phosphate dehydrogenase. *FEBS. Lett.* **134**, 143–146.
- Wei L., Alhenc-Gelas F., Corvol P. and Clauser E. (1991), The two homologous domains of human angiotensin I – converting enzyme are both catalytically active. *J. Biol. Chem.* **266**, 9002–9008.
- Tso J. Y., Sun X. H., Kao T. N., Reece K. S. and Wu R. (1985), Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: genomic complexity and molecular evolution of the gene. *Nucleic Acid Res.* **13**, 2485–2501.