ELSEVIER

Journal of Chromatography A, 853 (1999) 185–188

JOURNAL OF CHROMATOGRAPHY A

Short communication

Analysis of angiotension-converting enzyme by capillary electrophoresis

Zak K. Shihabi*

Pathology Department, Wake Forest University Baptist Medical Center, Winston-Salem, NC 27157, USA

Abstract

A method is described for determination of serum angiotension-converting enzyme by capillary electrophoresis (CE) based on incubation of the substrate, a synthetic peptide, with the serum outside the capillary and cleaving hippuric acid and a dipeptide. The reaction is stopped by the addition of acetonitrile, followed by injection of the supernatant on the capillary. The acetonitrile allows injection of a large volume of sample on the capillary. Both the substrate and the reaction product (hippuric acid) can be monitored at the same time. The CE step is rapid and can be performed in about 6 min. The CE method compared well to a kinetic assay method (=0.98).

Keywords: Angiotension-converting enzyme; Enzymes; Peptidases

1. Introduction

The angiotension-converting enzyme (ACE, dipeptidyl carboxypeptidase) is a glycoprotein peptidase that catalyzes the cleavage of a dipeptide histidylleucine from the substrate angiotension I to form the vasoconstrictor octapeptide angiotension II and also inactivates bradykinin. Thus, ACE is considered an important enzyme in regulating the blood pressure. Many drugs lower blood pressure by inhibiting this enzyme.

Clinically the serum level of ACE is used to diagnose patients with active sarcoidosis; a disease which is very difficult to diagnose by other methods. Furthermore, the disease responds well to steroid treatment.

Different synthetic peptides have been used for the analysis of ACE releasing chromogenic small peptides, which can be monitored easily by their optical absorption. The analysis of ACE has been performed by several methods including spectrophotometric [1,2], fluorescence [3,4] and HPLC [5,6].

Previously, using the enzymes cathepsin D [7] and *N*-acetyl-β-glucosaminidase [8], we demonstrated that CE is well suited for the analysis of enzymes in general and peptidases in particular. The latter group of enzymes acts on charged peptides which absorb light in the uv range. We have demonstrated that incubation of the enzyme with the substrate outside the capillary, followed by stopping the reaction with acetonitrile, enables the injection of a large sample volume on the capillary without band broadening [7]. Here, we describe that the CE can be used to determine the ACE activity in serum using the synthetic peptide hippuryl-histidyl-leucine as a substrate and acetonitrile for stacking.

*Tel.: +1-336-716-2639; fax: +1-336-716-9944.
E-mail address: zshihabi@wfubmc.edu (Z.K. Shihabi)

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00474-4

2. Experimental

2.1. Chemicals

Hippuryl-histidyl-leucine and furylacryloyl, phenylalanylglycylglycine were obtained from Sigma (St. Louis, MO, USA). Accupril was obtained from Park-Davis (Morris Plains, NJ, USA).

2.2. Electrophoresis buffer

Boric acid 135 m*M* adjusted to pH 8.4 with 2 *M* NaOH.

2.3. Substrate

Hippuryl-histidyl-leucine, 1 mg/ml, dissolved in Tris 80 m*M* pH 8.1 containing NaCl 9 g/l (stored refrigerated).

2.4. CE instrument

A Model 4000 Quanta (Waters Associates, Milford, MA, USA) was set at 12 kV and 254 nm (except where indicated otherwise). The sample was introduced hydrodynamically on a 30 cm×50 μm (I.D.) capillary and electrophoresed for 6 min. The capillary was washed for 0.5 min with 0.2 *M* NaOH and filled with the electrophoresis buffer for 1.0 min.

2.5. CE procedure

Serum (25 μl) was mixed with 100 μl of the substrate and incubated at 37°C for 20 min. To stop the reaction, acetonitrile (200 μl) was added to the incubation mixture, mixed and centrifuged at 15 000 *g* for 10 s. The supernatant was injected directly on untreated silica capillary for 120 s filling about 10% of the capillary volume.

2.6. Kinetic assay

For comparison purposes the CE method was compared to a kinetic spectrophotometric method based on the cleavage of the synthetic peptide furylacryloyl, phenylalanylglycylglycine (FAPGG) [2]. In this method 25 μl serum is reacted with 250 μl of the peptide and the reaction is pre-incubated for 3 min and monitored kinetically at 37°C at 340 nm for 6 min in a fully automated instrument (Opera, Bayer Corp., Tarrytown, NY, USA). The instrument calculates the unknown values from commercial secondary calibrators.

3. Results and discussion

Capillary electrophoresis is well suited for the analysis of peptides and the different enzymes involved in their metabolism. In this analysis ACE cleaves hippuric acid (*N*-benzoylglycine) and a dipeptide from the synthetic tripeptide hippuryl-histydyl-leucine releasing hippuric acid which absorbs light in the ultraviolet region. The maximum of absorption of hippuric acid is about 230 nm. The enzymatic activity can be monitored at 229 (Zn lamp) where both the substrate and products can be detected, Fig. 1. It can be seen that there is a decrease in the substrate (P) equivalent to the increase in the hippuric acid peak (H). The increase in hippuric acid peak, the decrease in the substrate or the ratio of the two can be used at this wavelength for calculation of enzymatic activity. Hippuric acid has also absorption at 254 nm which is about four times less than at 230. Although less sensitive, the 254 nm is a more common wavelength on most instruments with less possibility of interferences from other unknown compounds. At this wavelength, the reaction product, hippuric acid (H) is monitored, Fig. 2. A trace of the hippuric acid occasionally can be detected in the blank upon prolonged storage of the substrate. The addition of an ACE inhibitor, accupril (Quinaril, 0.5 mg/ml) to the reaction mixture inhibited the activity indicating that this reaction is specific.

Fig. 3 illustrates that the reaction based on sample dilution is linear to about 200 U/l and also linear with incubation time up to 45 min, Fig. 4. The recovery of hippuric acid added to the enzymatic reaction mixture is 99%, ($n=3$) relative to that added to the buffer. The minimum detection activity at 254 nm is about 10 U/l.

Converting the peak height to enzymatic activity (U/l) can be accomplished through transformation of

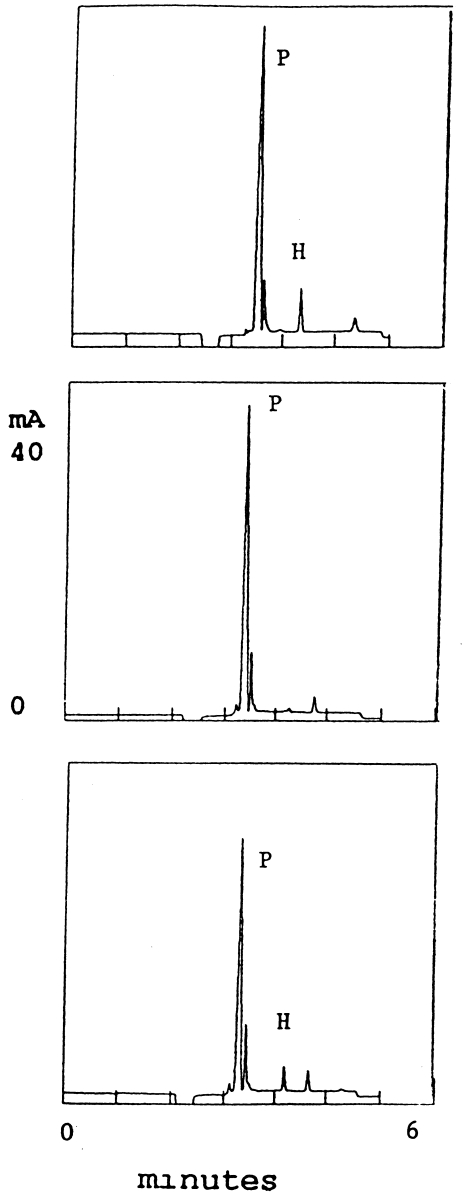

Fig. 1. (Top) Hippuric acid, H (1100 μ*M*); (middle) a serum sample (65 U/l) of a patient before incubation; and (bottom) same serum sample after incubation for 20 min at 37°C with detection at 229 nm (P, substrate, H, hippuric acid).

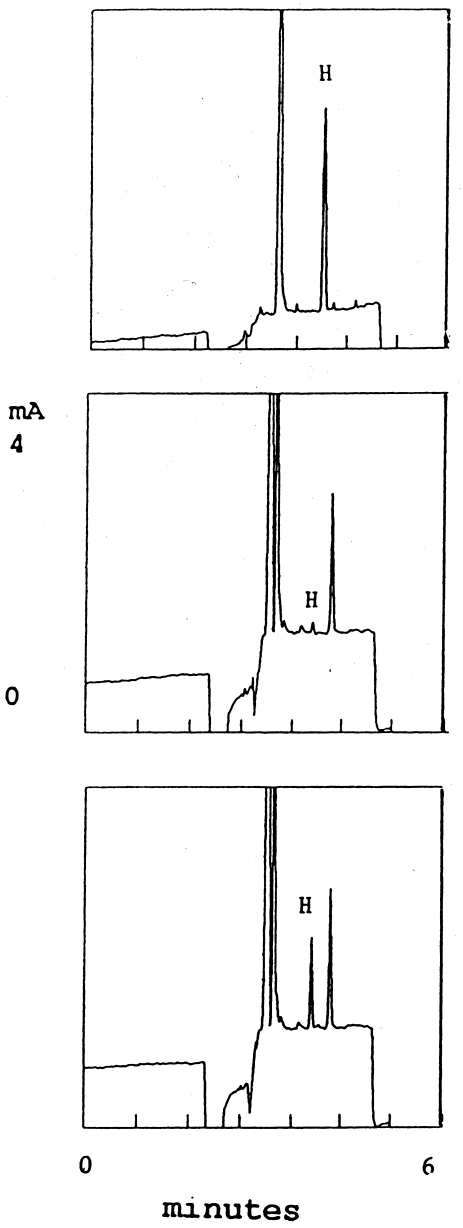

Fig. 2. (Top) Hippuric acid, H (1100 μ*M*); (middle) a serum sample (65 U/l) of a patient before incubation; and (bottom) same sample after incubation for 20 min at 37°C with detection at 254 nm.

the concentration of the hippuric acid to U/l directly or through comparison of the peak of the patient to that of secondary calibrators i.e. samples with known activity. The second approach is more suitable in this case. It overcomes many practical problems such as inaccuracy of reaction temperature or volumes. The reference interval for serum level is 10–50 U/l. Patients with active sarcoidosis tend to have elevated levels. The comparison of this method to an auto-

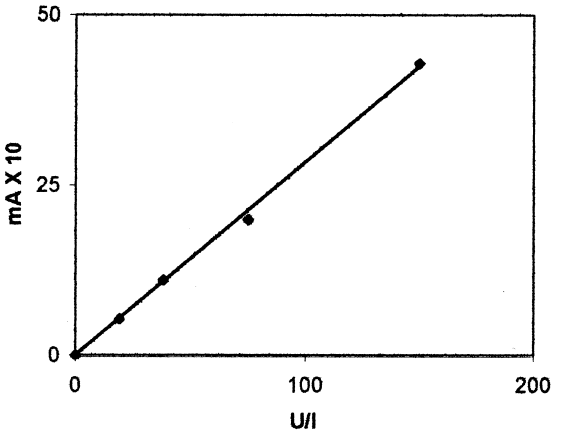

Fig. 3. Enzymatic activity as function of sample concentration.

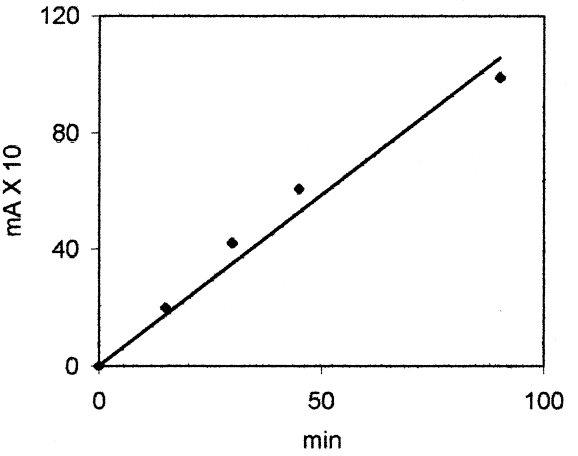

Fig. 4. Linearity of the assay with progress of time.

mated kinetic method [2] is very good $r=0.98$, $n=14$ (Fig. 5).

The use of acetonitrile in this method has these several advantages: it stops the reaction, eliminates the proteins and causes sample stacking; allowing injection of up to 10% of the capillary volume with the sample resulting in an increase in detection.

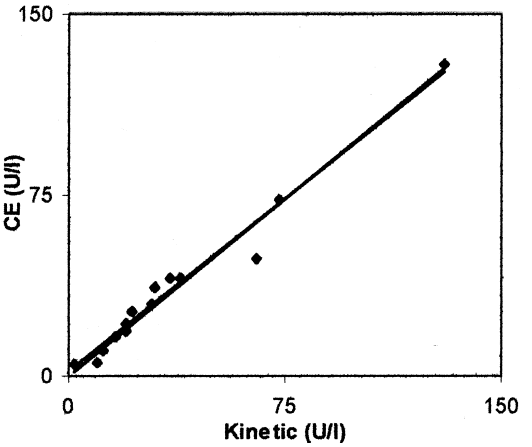

Fig. 5. Comparison of CE to a kinetic assay [2], $r=0.98$.

References

[1] M. Hayakari, Y. Kondo, H. Izumi, Anal. Biochem. 84 (1978) 361.
[2] B. Holmquist, P. Bunning, J.F. Riorden, Anal. Biochem. 95 (1979) 540.
[3] E. Maita, Y. Endo, Y. Ogura, Anal. Biochem. 128 (1983) 36.
[4] J.M. Conroy, C.Y. Lai, Anal. Biochem. 87 (1978) 556.
[5] M. Horiuchi, K. Fujimura, T. Terashima, T. Iso, J. Chromatogr. 233 (1982) 123.
[6] Z.K. Shihabi, J. Scaro, Clin. Chem. 27 (1981) 1669.
[7] Z.K. Shihabi, T. Kute, J. Chromatogr. B 683 (1996) 125.
[8] M. Freidberg, Z.K. Shihabi, J. Chromatogr. B 695 (1997) 187.