Est. 1984

ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2017, Vol. 33, No.(5):
Pg. 2676-2680

Leucine Aminopeptidase from *Arachis hypogaea* L. Seeds Partial Purification and Characterization

TAGHREED U. MOHAMMD*[1], LAYLA O. FARHAN[2], ASHGAN S. DAWOOD[2] and BUSHRAF. HASAN[2]

[1]Department of Chemistry, College of Education for Pure Sciences, Ibn-AL-Haitham-University of Baghdad, Baghdad, Iraq.
[2]Department of Chemistry, College of Science for Woman, University of Baghdad, Baghdad, Iraq.
*Corresponding author E-mail: tagreedaloom@gmail.com

http://dx.doi.org/10.13005/ojc/330567

(Received: July 19, 2017; Accepted: August 14, 2017)

ABSTRACT

Leucine amino peptidases (LAP; EC 3.4.11.1) constitute a diverse set of exopeptidases that catalyze the hydrolysis of leucine residues from the amino-terminal of protein or peptide substrates, (LAP) are present in animals, plants, and microbes. In this study, leucine amino peptidase was purified partial from A*rachis hypogaea* seeds by using gel filtration chromatography *Sephadex* G-100. The enzyme was purified 3.965 fold with a recovery of 29.4%. Its pH and temperature optimum were(8.7) and (37°C), respectively. The results show novel properties of LAP from *Arachis hypogaea* L. or peanut. The Km value for LAP (77 mM), with V max (1538 m mole min^{-1}). We recommend a separate isoenzymeof the enzyme (LAP) from *Arachis hypogaea on* L. peanut seeds and study the kinetic qualities of each of them

Keyword: Leucine amion peptidase (LAP), *Arachis hypoaea* L. seeds, Purification.

INTRODUCTION

Arachish ypogaea L. (Peanut) is one of seeds that has great quantity of oil and protein, therefor, it is considered as very important food legume to human nutrition in the world. We can find *Arachish ypogaea L. (Peanut)* widely distributed in the tropical and subtropical areas of the world[1]. It is an annual herbaceous plant growing (30-50) cm (1.0-1.6) feet tall. It has opposite leaves , pinnate with four leaflets (two oppositepairs; no terminal leaflet). The flowers of *Arachis hypogaea L.* are similar to typical pea flowers in shape, its color is yellow with reddish veining[2]. Biochemical, physiological, chemotaxonomy and gentic variability investigation studies make use of qualitative and quantitative isoenzymatic analysis. These studies benefit of a large number of plant population, cultivars and species[3].

Aminopeptidase (EC 3.4.11) is one of the group of proteases, important enzymes that play major role in many different life processes. The hydrolysis of amino acids can be catalyzed by using

Aminopeptidase which found in the N-terminal of peptide and are involved in proteins degradation to free amino acids[4]. The major and greatest studied group of amino peptidase is Leucin amino peptdase (LAP, EC 3.4.11.1), especially in microorganisms and animals, therefor recognize it is x-ray crystal structure and their nucleotide sequences[5].

Generally, two classes of leucyl amino peptidases (LAP_s) have been reported for most plant species[6]. The first group has ther molabile amino peptidases with molecular weight of approximately 60-90 KDa and a neutral pH optimum. The second group are enzymes like plant LAP_s but isolated from animals. With large (250-330 KDa), homohexamer icmet all opeptidases that contain ethylene diaminetetraacetic acid (EDTA) and be statin. They are heat stable and possess on alkaline pH optimum. LAP_s with alkaline pH optimum have been biochemically purified from a number of plants[7]. In the present study, we purified major LAP from *Arachis hypogaea* L**.** seeds and characterized its enzymoloical properties (pH and temperature optimum).

MATERIAL AND METHODS

Enzyme Extraction

Arachis hypogaea L. (Imported from Turkey)was obtainedfrom a local super market in Baghdad, Iraq. Homogenizing 1 g homogenate at 18000 g for 30 of *Arachis hypogaea* seeds in 5 ml of phosphate buffer, pH 7.8 use a Teflon pestle homogenizer were used for preparing crude extract. The supernatant was separated as crude after getting rid of insoluble debris by centrifuging min. at 4°C .

Detrminations of protein concentration

This method is based on the reaction of proteins with the alkaline sulphate followed by Folin-cicalteau reagent which produces a blue color complex due to the reaction of alkaline copper sulphate with the protein, the intensity of the color depends upon the quantity of the protein detected[8]. As shown in Figure. (1).

Determination of LAP activity

hydrolysis of the peptide bond of leucinamide is measured according to Mitz and Schlueter method spectrphotometrically at 238 nm. Unit of enzyme activity like one micromole of L-lucinamide hydrolyzed per minute at 25°C and pH 8.5[9].

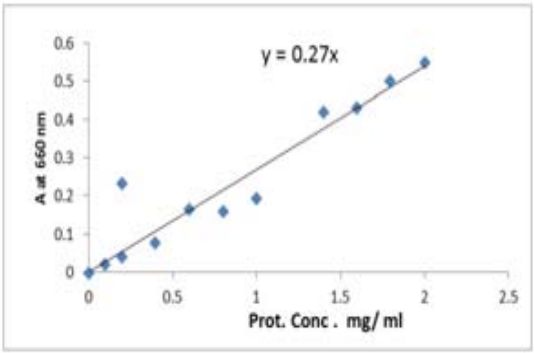

Fig. 1. Protein standard curve

Parital purification of LAP

Gel filtration chromatography using *sephadex* G-100 column was used for purification of partially purified enzyme. The height of 120 cm in a glass column with an internal diameter of 2.0 cm were used for packing the column ,then equilibrated with 0.1 MT ris-HCl buffer pH 8.5 .The flow rate was at 0.5 ml min^{-1}. Forty fractions had been collected for each 2 ml and both the protein content and the enzyme activity were determined for each separate fraction, as pointed out in the previous section.

Effect of pH on LAP activity

The effect of pH on LAP activity in the purified extract was firmed in different pH (8.1, 8.3, 8.5, 8.7, 8.9) with Tris-HCl buffer pH 8.5. Then LAP activity was measured.

Effect of temperature on LAP activity: Theeffect of temperature on LAP activity was firmed in different temperature (20, 24, 37, 40, 45°C) with Tris-HCl buffer pH 8.5. Then LAP activity was measured.

Different concentration of substrate

Different concentration have been prepared (45, 85, 125 , 165, 205) m M /l of substrate (leucinamide) in buffer[9] Km and Vmax for enzyme to substrate were determined by using the Lineweaver-Burk plot [the relationship between 1/V versus 1/[S].

RESULT AND DISCUSSION

Table 1 summaries the result of partial purification of LAP from *Arachis hypogaea*. The supernatant with LAP activity of 1486.0 Unit/mL and specific activity of 413.23 Unit/mg was considered as crude enzyme solution. This crude enzyme solution fraction was made up to a known volume, partially purified LAP was obtained from this crude enzyme solution fraction and loaded on *sephadex* G-100 gel filtration column.Gel filtration was purified to the enzyme 3.965 fold with a yield 29.4% and specific activity of 1638.75 Unit/mg. as shown Figure. (2).

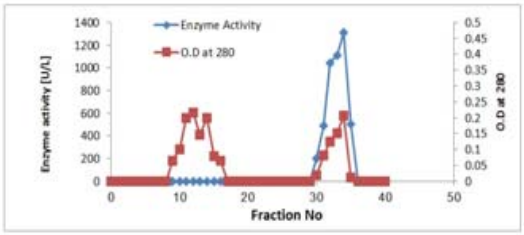

Fig. 2. Atypical elution profile for the chromatography leucin-aminopeptidase from *Arachis hypogaeaon L.* using *Sephadex* G-100

The exact role of LAP_s in plants is not known, while it is enzymes take part in some important processes, such as protein mobilization from cotyledons after germination, and protein turnover required for cell maintenance in vegetative and reproductive organs[10]. LAP_s are involved in rapid turnover of protein required in wounding or wounding or pathogen attack[11].

The highest enzyme activity in the range of pH (8.1 - 8.9), with optimum activity in Tris-HCl buffer at pH 8.7 was shown as in figure (3). This result was agree with the value got from *Fasciola gigantic* LAP at pH 8.0[12], from Kiwifruit pH 9.0[13]. In comparison with , aromatic aminopeptidase which was isolated from grapes gave a result which was pH 7.0[14], from barley pH 7.2[15] and from wheat pH

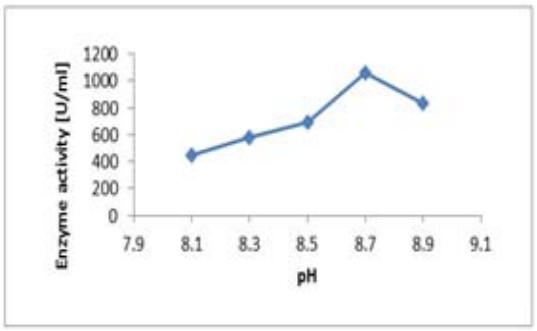

Fig. 3. Effect of pH on LAP activity

7.6[5]. While Matsui *et al.,* 2006[9] showed that maximum activity was obtained at alkaline pH (8.0-11.0).

The optimum temperature for the activity of LAP from *Arachis hypogaea L*. seeds was determined to be 37°C. as shown in Fig. (4). This result like the value obtained from pea shoots 37°C[16,17]. In the other studies kiwifruit AP was most

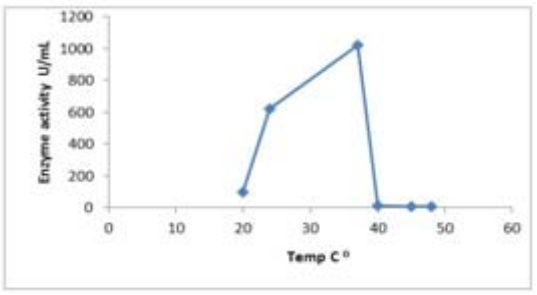

Fig. 4. Effect of temperature on LAP activity

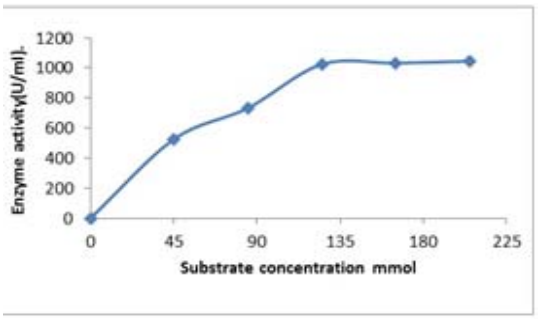

Fig. 5. Activity of LAP with different

Table. 1: Purification summary of aminopeptidaseArachis hypogaea.

	Volume (ml)	Activity (Unit/ml)	Total activity (units)	Total protein (mg/g)	Specific activity (Units/mg.)	Fold purification	Recovery
Crude enzyme	6	1486.0	8916	3.596	413.23	1	100
Sephadex G-100	2	130107	2621.4	1.6	1638.75	3.965	29.4

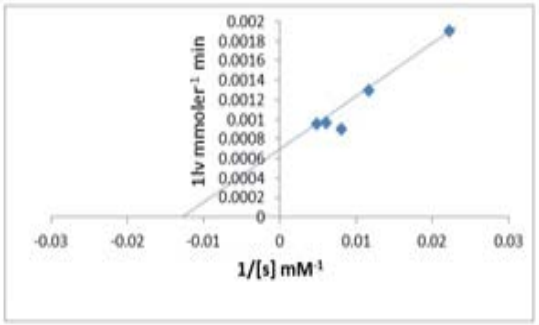

Fig. 6. Determination of Km and Vmax in partial purified enzyme .

active at 37°C[13]. Unlike stability of aromatic aminopeptidase, lucine aminopeptdases remained even at temperatures over 60°C, an optimum temperature of 60-70 °C was used for characterizing their activities[9].

A linear relationship was obtained a Vmax [1538 U/mL] and Km value of [77 mM].An enzyme with low Km has a more affinity for its substrate .

CONCLUSION

In conclusion , in the present study,it was found one peak of the *Arachishypogaeaon L*.peanut enzyme (LAP) by chromatography(gel filtration).The *Arachi shypogaeaon L*. peanut enzyme (LAP) reach optimum activity at pH is 8.7 and temperature at 37°C . The purified enzyme indicated maximum activity (Vmax) of 1538 m mole min^{-1} with its parallel Km value of 77 Mm. We recommend a separate isoenzyme of the enzyme (LAP) from *Arachis hypogaeaon L.* peanut seeds and study the kinetic qualities of each of them . Note that the enzyme was purified for the first time and have got a successful and appropriate way because we got a good yield.

REFERENCES

1. Maria, L., and Catalina. R.L. .Isoenzimatic variability among five peanut cultivars. Bragantia.Campinas., **1994,** *53(2)*,135-140.
2. Ryals , J.; Ward, E.; Ahl-Goy, P., and Metraux, J. P. . Inducible plant proteins: Society for Expermintal Biology Series, ed. Wary, *J. L. (Cambridge Univ. Press*, New York), **1992,** *49* ,205-229.
3. Cherry, J.P., and Ory, R.L. . Electrophoretic characterization of six selected enzymes of peanut cultivars. *Phytochemistry*, Oxford, **1973,** *12(7)*, 283-289.
4. Joana, K., and Danuta. G. .Aminopeptidases isolated from plants of great economic value-role and characteristics. Chemik., **2015**, *69(8)*, 463-468.
5. Magda, P.; Urszula, S.;Wieslaw, B. and Edyta, Z. . Purification, biochemical characterization, and mass spectrometry analysis of phenylalanine aminopeptidase from the shoots of pea plants.*Acta Physiol. Plant.*, **2011,** *33*, 609-617.
6. Zoran, V.; Bilijana, D.; Aleksandra, M. and Natasa, B. .Purification and Properties of the Major Lucyl aminopeptidase from Solanum tuberosum Tubers.*Fruit, Vegetable and Cereal Science and Biotechnology*. Special Issue, **2008.** *1*., 125-130.
7. Matsui, M.; Fowler, HJ. and Walling, LL. . Lucine aminopeptidase: *diversity in structure and function. Biological Chemistry*., **2006,** *387*, 1535-1544.
8. Sadasivam, S. and Manikam, A. . In: *Biochemical Methods for Agricultural Science*, Wily Eastern Limited, New Delhi and TNAU, Coimbatore., India, **1992**, 122-123.
9. Mitz, M. and Schlueter, R. Direct Spectrophotometric Measurement of the Peptide Bond: Application to the Determination of Acylase 1. *Biochim. Biophys. Acta*, **1958,** *27*, 168.
10. Park, SY. And Walling, LL.Isolation and characterization of the neutral leucine aminopeptidase (LapN) of tomato. Plant Physiology., **2003** *132(1*), 243-255.
11. Gu, YQ. and Walling, LL. Specificity of the wound- induced leucine aminopeptdase (LAP-A) of tomato activity on dipeptide and tripeptide substrates. *European Journal of Biochemistry*, **2000,** *207(4)*,1178-1187.
12. Daniel, C.; Fernando G. and Carlos, C. Characterizayion and Partial Purification of a Leucine aminopeptidase From *Fasciola*

hepatica. J. Parasitol., **1998***, 84(1),*1-7.

13. Permarathne, A.A. and David, W.M. Characterization of Activity of a Potential Food-Grade Lucine aminopeptidase from Kiwifruit. Enzyme Research, Articale ID, **2010**, *51*, 7283,1-5.
14. Mahagamasekera, M.G.P. and Leung, W.M. . Development of leucine aminopeptidase activity during dalily flower growth and senescence.*Acta Physiologiae Plantarum*, **2001,** *23(2)*, 181-186.
15. Kang, H. C.; Hahn,T.-R.; Chung,I-S. and Park, J-C. Characterization of aminopeptidase from grapes. *Int J Plant Sci*., **1999,** *160* ,299-306.
16. Oszywa,B.; Makowski, M. and Pawelczak, M. . Purification and partial characterization of aminopeptidase from barley(Hordeum vulgare L.)seeds. Plant Physiol. Bioch., **2013,** 65,75-80.
17. Ogiwara, N.; Amano, T.; Satoh, M. and Shioi, Y. . Lucine aminopeptidase from etiolated barley seedling: characterization and partial purification of isoforms. *Plant Sci*., **2005,***168,* 575-581.