Asian Journal of Chemistry Vol. 21, No. 7 (2009), 5104-5116

Carbonic Anhydrase from Potato (*Solanum tuberosum*) Roots and Leaves

NAZAN DEMIR*, HAYRUNNISA NADAROGLU† and YASAR DEMIR‡
Department of Chemistry, Faculty of Sciences, Atatürk University, 25240 Erzurum, Turkey
Fax: (90)(442)2360948; Tel: (90)(442)2314439
E-mail: demirn@yahoo.com

Carbonic anhydrases (CA: carbonate hydrolase: E.C.4.2.1.1) from leaves and roots of mature potato (*Solanum tuberosum*) were purified and characterized. The purification levels of enzymes were 87.91 fold and 40.601 fold in leaves and roots, respectively. The optimum temperature was 40 ºC in leaves and in roots. pHs optimal were 8.5 and 11 in leaves and in roots, respectively. Enzymes of leaves were formed 5 monomers that it's having molecular weights of 22,000, 28,000, 35,000, 40,000, 65,000, 5 polymers that it's having 73,000, 80,000, 83,000, 132,000 and 200,000 Dalton and these proteins had carbonic anhydrase activity. But there is at the levels of 22,000, 28,000, 35,000 and 132,000 Dalton in gel filtration, for leaves. SDS-PAGE was done for roots and leaves and subunits were obtained. In addition the enzyme against the effect of NaN_3, KSCN and sulphanylamide, which was known as an inhibitor of mammalian carbonic anhydrase, was determined. Potato (*Solanum tuberosum*) has ascorbic acid (vitamin C) in very high level and iron and calcium ions in low level. So, the effect of ascorbic acid, $FeCl_3$ and $CaCl_2$ in different concentrations on enzyme was determined for roots and leaves, separately. Finally, carbonic anhydrase was purified from roots and leaves of potato (*Solanum tuberosum*), separately and they were done optimal. Carbonic anhydrase functions in respiration and has a part in photosynthesis in plants. It was a serious deficiency that carbonic anhydrase wasn't defined in potato (*Solanum tuberosum*).

Key Words: Potato, *Solanum tuberosum*, Potato carbonic anhydrase.

INTRODUCTION

Carbonic anhydrase (CA; EC 4.2.1.1) catalyzes the reversible hydration of CO_2, accelerates the bicarbonate formation and plays an important role in several physiological functions in mollusks, including acid-base regulation, respiration, calcification and mineralization[1]. The activity of carbonic anhydrase and its influence on physiological functions in mollusks have been extensively investigated[1-4]. Carbonic anhydrase was demonstrated to play a role in mineralization of all invertebrates studied and was found to be essential for rapid shell development.

†Department of Food Technology, Oltu Vocational Training School, 25400, Oltu, Erzurum, Turkey.
‡College of Education, Chemistry Department, Atatürk University, 25240, Erzurum, Turkey.

The enzyme of carbonic anhydrase was purified and characterized in parsley, tea, *Daucus carota* and *Vicia canenses*[5-8].

The carbonic anhydrases from higher plants exists at least two electrophoretically separable types. One type, found predominantly in monocotyls, has a molecular weight of 40,000 Dalton[5]. The other, isolated from dicotyls, is a hexameric enzyme with a molecular weight of 180,000 Dalton and contains 6 tightly bound zinc ions[9].

Until now carbonic anhydrase of potato (*Solanum tuberosum*) that was a very important plant wasn't been defined. So, this study was planed in two parts. In the first part, enzyme purified and characterized from leaves and roots, separately and their properties compare with different plant and mammalian carbonic anhydrase. In second part of this study, it was aimed that the effect of NaN_3, KSCN, sulphanylamide, ascorbic acid, $FeCl_3$, $CaCl_2$ on carbonic anhydrase of potato (*Solanum tuberosum*) which is included a lot of ascorbic acid and a little iron and calcium ions was determined.

EXPERIMENTAL

Extract preparation: Potato (*Solanum tuberosum*) was collected from east Anatolia region of Turkey and then leaves and roots of this plant were separated mechanically. They were stored at -31 ºC. Carbonic anhydrase was purified from leaves as described earlier by Demir *et al.*[7].

Enzyme purification: The enzymes present in extracts were purified with ion exchange chromatography on 3 × 50 cm column that contained DEAE-Cellulose. Elution was carried out with 0.2 M *tris*-acetate and 0.01 M β-mercaptoethanol, at pH: 7.0.

Protein determination: Absorbency of obtained elutions was measured at 280 nm and amount of protein in elutions was determined[10].

Enzyme activity determination: Esterase activity was determined as described by Verpoorte *et al.*[11]. For this procedure, 1.5 mL of a buffered enzyme solution (0.1 mL enzyme, 1.4 mL 0.05 M *Tris*-SO_4, pH:7.4) and 1.5 mL of substrate were mixed in a cuvette and 3 min later, the absorbance was measured (348 nm, 25 ºC). A blank measurement was obtained by preparing the same cuvette without the enzyme solution. V_{max}, K_m, optimal pH and optimal temperature were determined by this method. V_{max} and K_m values were determined from Lineweaver-Burk graph. The hydrolysis activities of the purified enzymes were measured by determining the amount of time necessary to change the pH from 8.2 to 6.3.

Enzyme units were calculated according to the formula:

$$1U = ((t_o-t_c)/t_c)$$

where t_o and t_c the time (s) needed for the pH change without enzyme and with enzyme reactions, respectively.

Effect of various some chemicals in enzyme activity: The effects of sulphanylamide, KSCN, NaN_3, which are known as inhibitors and glucose, fructose and saccharose in three different concentrations on carbonic anhydrase that was purified from

roots and leaves were measured. These measures were done by using hydratase activity of enzyme[12].

SDS-PAGE Electrophoresis: Electrophoresis was carried out 3-10 % SDS-PAGE gel as described by Laemmli[13]. Human carbonic anhydrase-I which were purified by affinity chromatography and were used as electrophoresis standards[13].

Determination of molecular weight: The molecular weights of purified carbonic anhydrase enzymes of potato (*Solanum tuberosum*) leaves and roots were determined by Sephadex-G 150. Mixture of standard proteins, which had a concentration of 0.2 mg/mL, was applied on this column. Then, purified carbonic anhydrase enzymes were added on to equilibrated above columns and they were eluted with 0.05 M sodium phosphate-1 mM dithioerythritol, pH 7.0 buffer[14].

RESULTS AND DISCUSSION

A very important part of this study was that the enzyme of carbonic anhydrase purified from roots and leaves of potato (*Solanum tuberosum*). The carbonic anhydrases of leaves and roots of potato were purified by DEAE-cellulose ion exchange chromatography.

The amount of purified carbonic anhydrase was slightly high in roots than in leaves. Esterase activity of carbonic anhydrase was detected with *p*-nitrophenyl acetate as substrate. In the present study, carbonic anhydrase of leaves was purified 87.91 fold and it was 40.601 fold in roots carbonic anhydrase (Tables 1 and 2). Besides, graph of activity-absorbance was drawn (Fig. 1).

TABLE-1
CARBONIC ANHYDRASE FROM POTATO *(Solanum tuberosum)* ROOTS

Enzyme fraction	Volume (mL)	Activity (EU/mL)	Total activity		Protein (µg/mL)	Specific activity (EU/mg)	Purification fold
			EU	%			
Crude extract	580	1.445	838.10	100	38.40	0.0376	–
$(NH)_2SO_4$ 120 g	585	1.128	659.88	78.735	33.20	0.0339	0.901
$(NH)_2SO_4$ 180 g	615	0.895	550.42	83.412	26.70	0.0335	0.988
After DEAE-cellulose column	75	1.237	17.77	3.229	0.42	2.9450	87.910

TABLE-2
CARBONIC ANHYDRASE FROM POTATO *(Solanum tuberosum)* LEAVES

Enzyme fraction	Volume (mL)	Activity (EU/mL)	Total activity		Protein (µg/mL)	Specific activity (EU/mg)	Purification fold
			EU	%			
Crude extract	580	14.45	8381.0	100	28.40	0.508	–
$(NH)_2SO_4$ 120 g	585	11.28	6598.8	78.735	23.20	0.486	0.956
$(NH)_2SO_4$ 180 g	615	8.95	5504.2	83.412	20.70	0.432	0.983
After DEAE-cellulose column	75	7.37	477.75	8.679	0.42	17.540	40.601

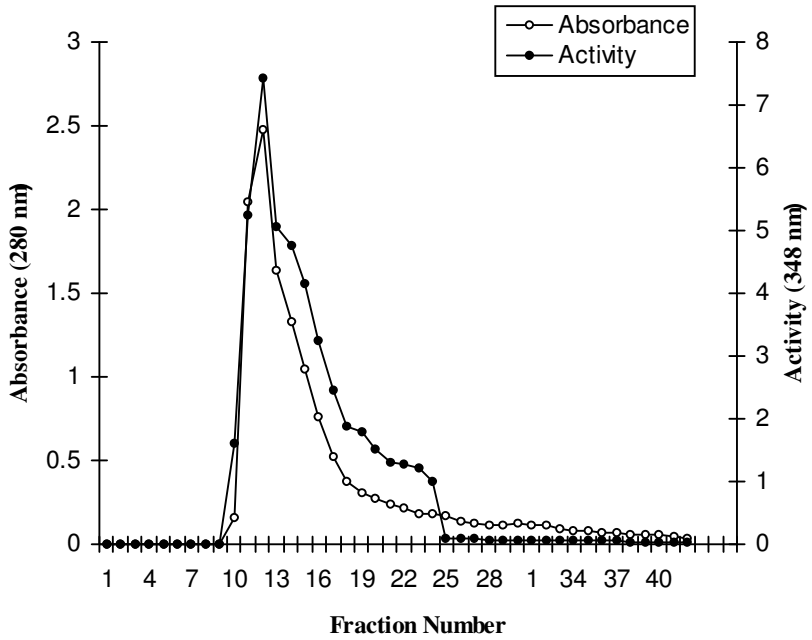

Fig. 1A. DEAE-cellulose ion-exchange chromatography of carbonic anhydrase from potato (*Solanum tuberosum*) roots in the presence of 0.2 M *Tris*-acetate buffer pH: 7.0, 0.01 M β-mercaptoethanol

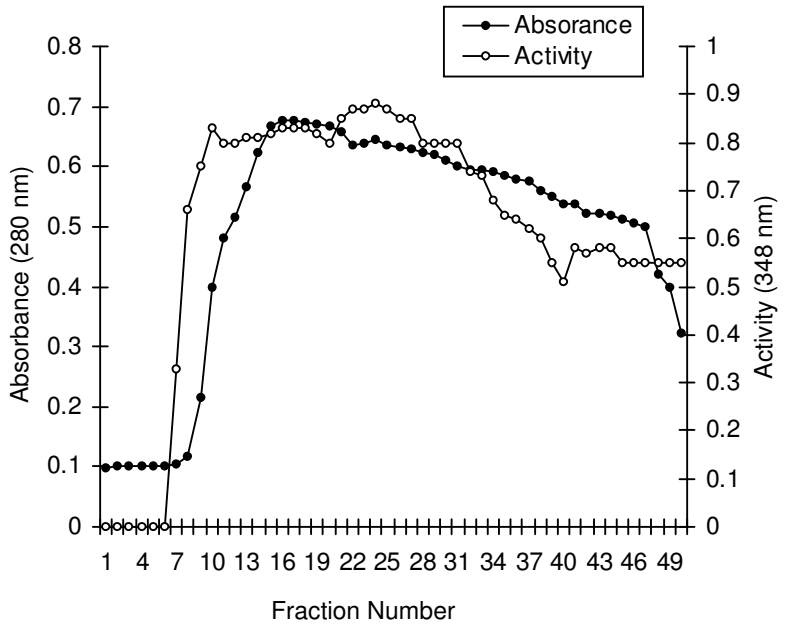

Fig. 1B. DEAE-cellulose ion-exchange chromatography of carbonic anhydrase from patato leaves in the presence of 0.2 M *Tris*-acetate buffer pH: 7.0, 0.01 M β-mercaptoethanol

As both carbonic anhydrase of leaves and roots of potatoes (*Solanum tuberosum*) had esterase activities, for each enzyme K_M and V_{max} values were determined which were 0.00232 mM and 2.120 mM/L min, respectively for leaves and 0.062 mM and 4.203 mM/L min, respectively for roots.

The optimum pH values of the carbonic anhydrases from potatoes (*Solanum tuberosum*) were 8.5 and pH 11 in leaves and in roots respectively (Fig. 2). pH of carbonic anhydrase of roots and leaves were different from each other.

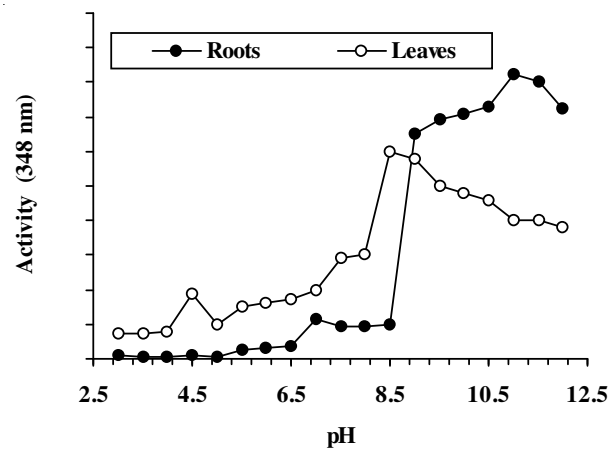

Fig. 2. Activity of carbonic anhydrase enzyme from potato (*Solanum tuberosum*) roots in *Tris*-acetate buffer

The optimum temperature for leaves carbonic anhydrase were 40 ºC in roots and leaves. The temperature range at which enzymes have activity was found as 5-85 ºC (Fig. 3).

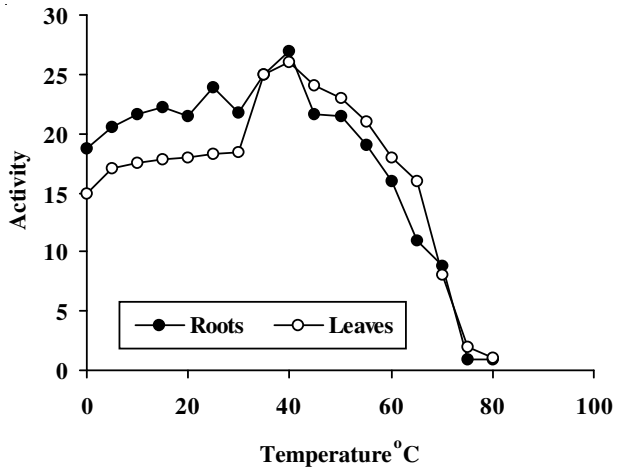

Fig. 3. Effect of temperature on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) roots

The molar mass of the roots and leaves carbonic anhydrases determined by gel filtration chromatography. The molecular weight of the roots determined as 22,000, 28,000, 35,000, 40,000, 65,000, 73,000, 80,000 and 83,000 Dalton and these proteins had carbonic anhydrase activity. These bands were shown on electrophoresis (Fig. 4). At the level of 40,000 Dalton, the protein was a lot of, as amount. Band was very bold at the level of 40,000 Dalton. When gel filtration was done, the highest activity was seen at fraction of level of 40,000 Dalton. According to results of gel filtration, there are two bands having molecular weights 132,000 and 200,000 Dalton and there are another two bands which weren't shown in SDS-PAGE and they also have carbonic anhydrase activity. They had probably subunit more than one. But it is difficult to say any thing about molecular weight of subunit. But the smallest subunit was the level of 22,000 and 132,000 was 6 fold of 22,000 and 200,000 was 9 fold of 22,000. Hexameric subunit was shown in plant[6-9].

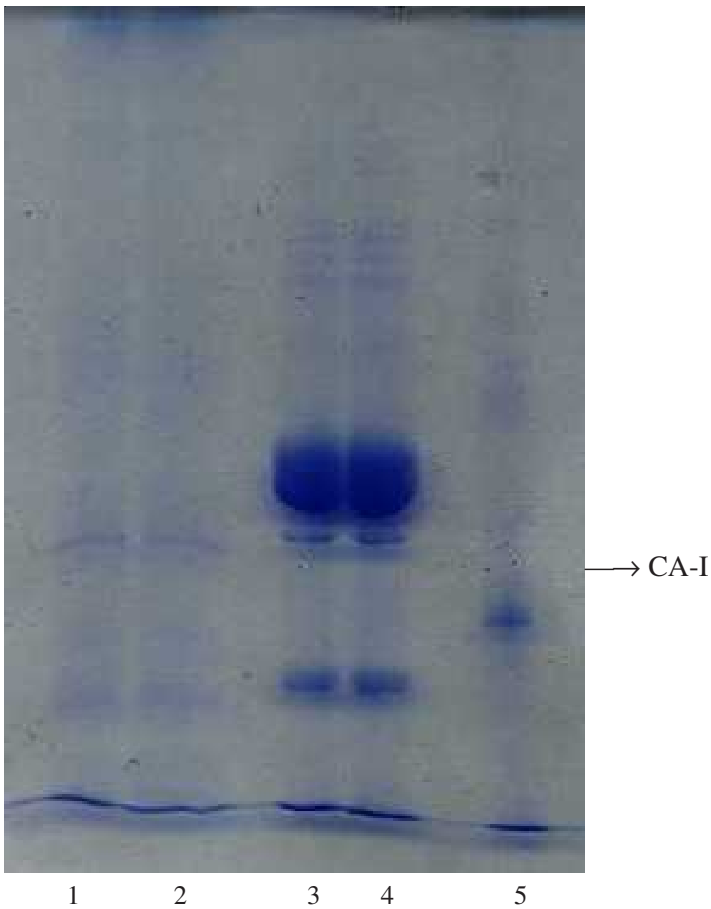

Fig. 4. Electrophoretic pattern of potato (*Solanum tuberosum*) carbonic anhydrases: leaves (1 and 2), roots (3 and 4) and carbonic anhydrase (CA)-I (5)

There are three bands at the level of 22,000, 28,000 and 35,000 Dalton on electrophoresis of roots carbonic anhydrase. But there was only one band the level of 132,000 Dalton in gel filtration. It can be accepted that hexameric structure with 6 subunit having molecular weights of 22,000 Dalton. At roots, another shown form were not found at leaves. These structure were similar to *Vicia caneces*' carbonic anhydrase. It had also 20,000 Dalton subunit.

NaN_3 was activated very little in 10^{-2} M concentration on roots and leaves carbonic anhydrase activity but 10^{-4} M and 10^{-6} M concentrations, the activity of roots' carbonic anhydrase and leaves' carbonic anhydrase against were ineffective (Fig. 5A and 5B).

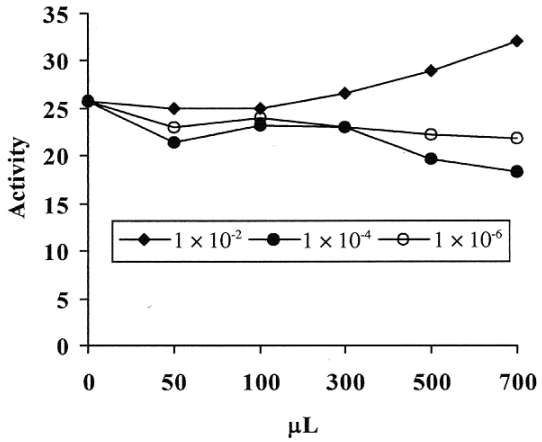

Fig. 5A. Effect of NaN_3 in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) roots

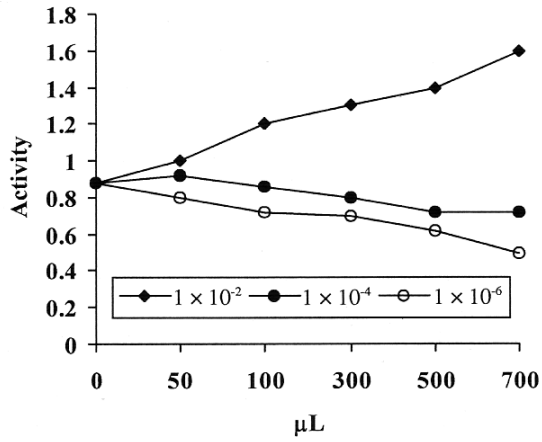

Fig. 5B. Effect of NaN_3 in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) leaves

For carbonic anhydrase of roots, a high activation was seen in each of three concentrations and 50 μL volume KSCN. When the effect of activation was decreased in increasing volume. Carbonic anhydrase of leaves was activated 10^{-2} M, 10^{-4} M, 10^{-6} M KSCN (Fig. 6A and 6B).

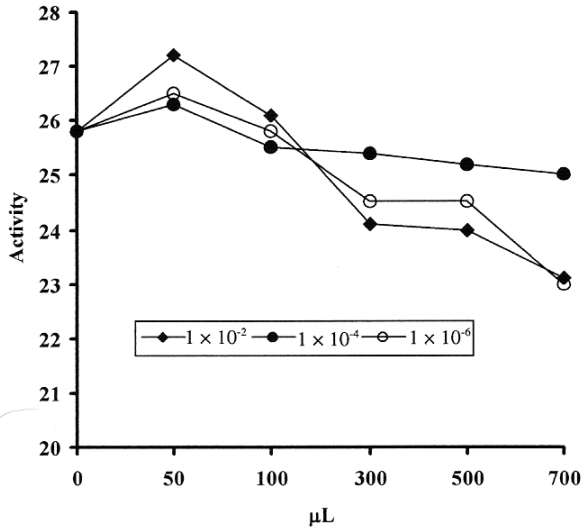

Fig. 6A. Effect of KSCN in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) roots

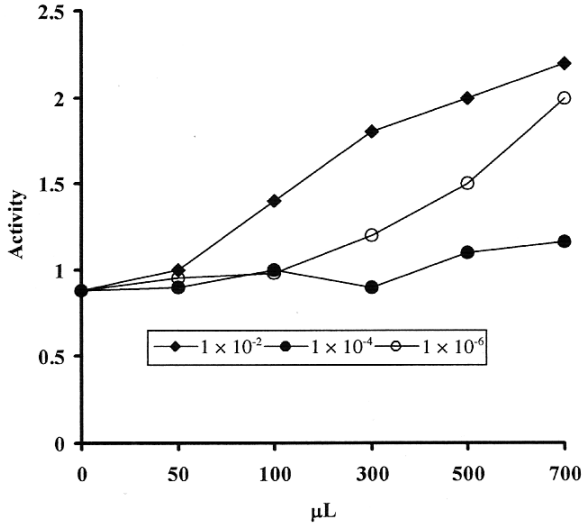

Fig. 6B. Effect of KSCN in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) leaves

Sulphanylamide was inhibited in potato roots and leaves carbonic anhydrase activity (Fig. 7A and 7B). Ascorbic acid was ineffective in potato roots carbonic anhydrase activity but it was activated in leaves carbonic anhydrase activity at three different concentrations (Fig. 8A and 8B).

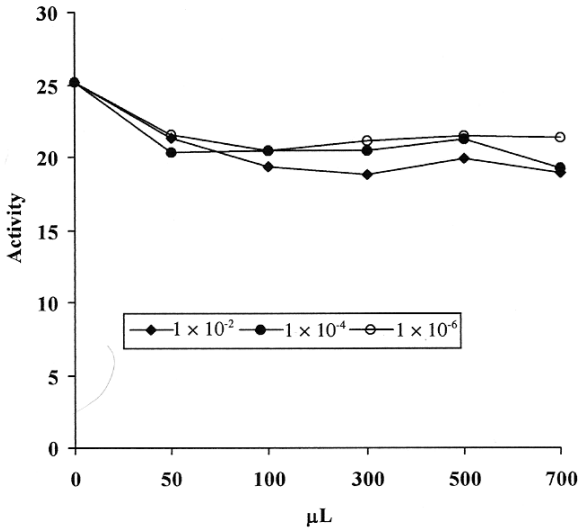

Fig. 7A. Effect of sulphanylamide in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) roots

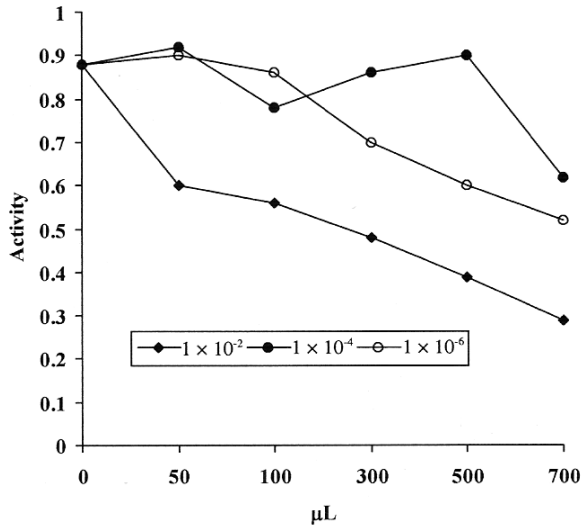

Fig. 7B. Effect of sulphanylamide in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) leaves

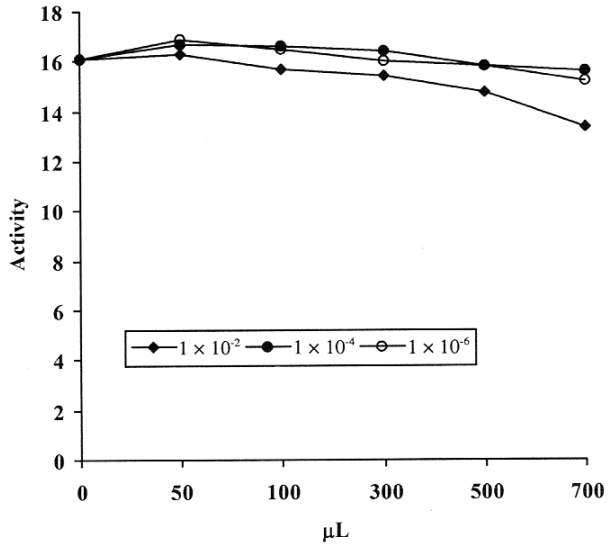

Fig. 8A. Effect of ascorbic acid in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) roots

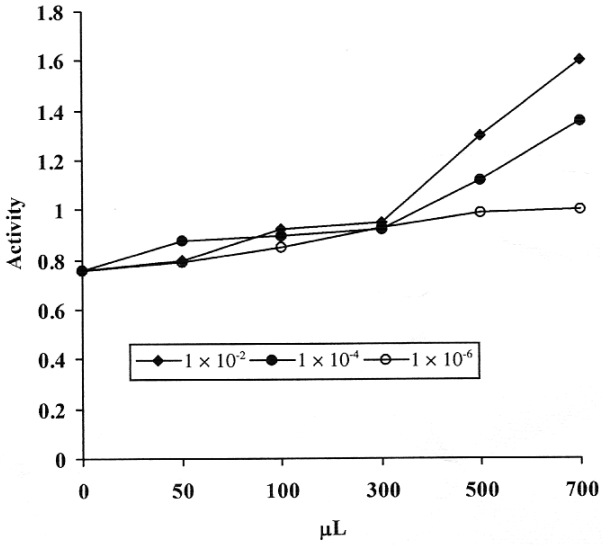

Fig. 8B. Effect of ascorbic acid in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) leaves

Other chemical that was studied was $CaCl_2$. It was seen that $CaCl_2$ was ineffective on roots carbonic anhydrase at three different concentrations (Fig. 9A and 9B).

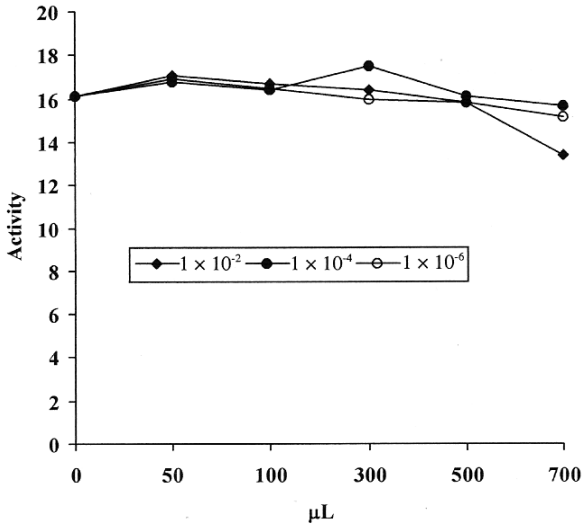

Fig. 9A. Effect of $CaCl_2$ in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) roots

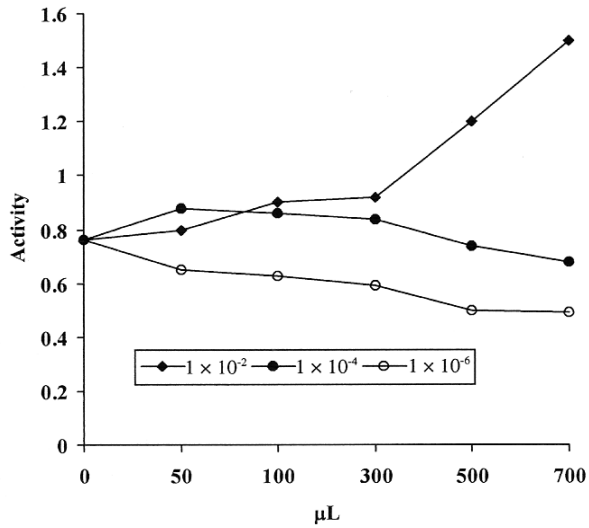

Fig. 9B. Effect of $CaCl_2$ in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) leaves

Finally, the effect of $FeSO_4$ at three different concentrations was studied, it was seen that they were ineffective on the activity of roots and leaves carbonic anhydrase. $FeSO_4$ wasn't inhibited or activated to the enzyme of carbonic anhydrase (Fig. 10A and 10B).

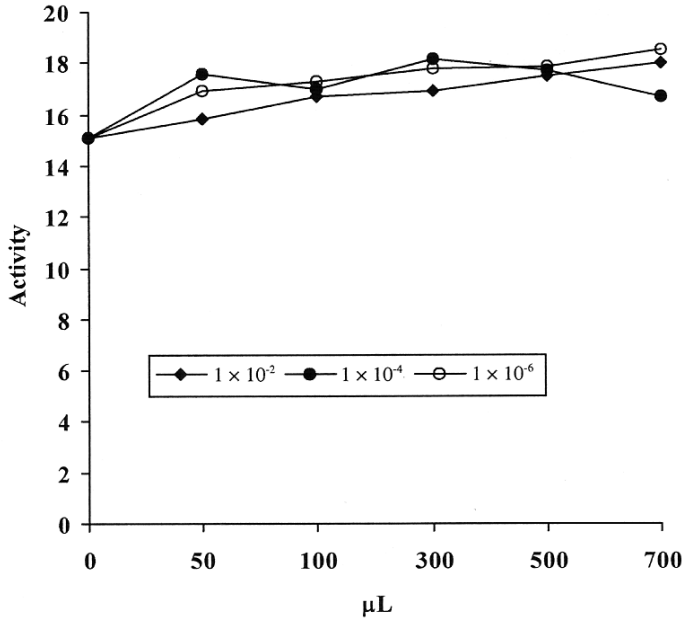

Fig. 10A. Effect of $FeSO_4$ in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) roots

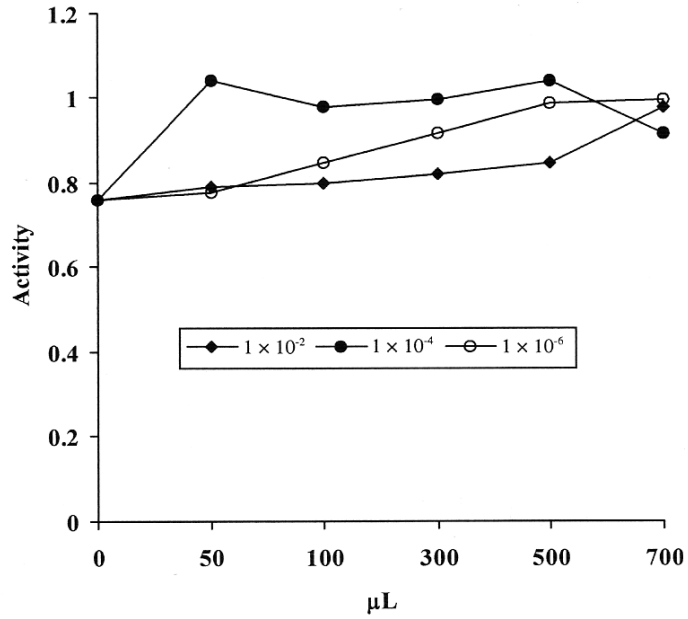

Fig. 10B. Effect of $FeSO_4$ in various concentrations on the activity of purified carbonic anhydrase enzyme from potato (*Solanum tuberosum*) leaves

In present studies, the carbonic anhydrase was purified and characterizated from potato. Carbonic anhydrase is an enzyme which function in respiration and take part in photosynthesis in plants. There is no report about the carbonic anhydrase from potato. Chemicals which were activated by carbonic anhydrase enzyme were increased photosynthesis and roots' greatness or they were inhibited, so carbonic anhydrase enzyme decreased, probably. Related to this, it can be done optima of cultivated environment. So, cultivated time can be shortened by the side of potatoes. Further researches are going on this subject.

REFERENCES

1. K.M. Wilbur and A.S.M. Saleuddin, in eds.: A.S.M. Saleuddin and K.M. Wilbur, Shell Formation, The Mollusca, Academic Press, New York, Vol. 4, pp. 235-287 (1983).
2. L. Duvail, J. Moal and M.F. Peron, *Comp. Biochem. Physiol. C*, **120**, 475 (1998).
3. D. Medakovic, *Helgol. Mar. Res.*, **54**, 1 (2000).
4. R.P. Henry, S. Gehnrich, D. Weihrauch and D.W. Towle, *Comp. Biochem. Physiol. A*, **136**, 243 (2003).
5. A.L. Tobin, *J. Biol. Chem.*, **245**, 2656 (1970).
6. N. Demir and Y. Demir, *Doga Turk. J. Chem.*, **21**, 111 (1997).
7. N. Demir, Y. Demir and G. Agar, *Prep. Biochem. Biotech.*, **27**, 271 (1997).
8. N. Demir, Y. Demir and A. Yildirim, *Phytochemistry*, **44**, 1247 (1997).
9. Y. Demir, N. Demir and Ö.I. Küfrevioglu, *Prep. Biochem. Biotech.*, **29**, 235 (1999).
10. H.P. Bradford, *Anal. Biolchem.*, **72**, 248 (1976).
11. J.A. Verpoorte, S. Mehta and J.T. Edsall, *J. Biol. Chem.*, **242**, 4221 (1967).
12. E.E. Rickli, S.A.S. Ghazanfar, B.H. Gibbons and J.T. Edsall, *J. Biol. Chem.*, **239**, 1065 (1964).
13. U.K. Laemmli, *Nature*, **227**, 680 (1970).
14. J.R. Whitaker, *Anal. Chem.*, **35**, 1950 (1963).

(*Received*: 19 April 2008; *Accepted*: 29 April 2009) AJC-7441