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Short Article

Effects of Zinc, Sodium and Potassium Metal Ions on Ammonium Fraction of Acid Phosphatase of Orange Fruit

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ABSTRACT

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Key words
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Backgrounds and Aims: The purpose of this study was to examine the effects of potassium, sodium and zinc metal ions on ammonium sulfate fraction of acid phosphatase activity of orange fruit.

Materials and Methods: Acid phosphatase was extracted and purified from orange fruit and then various samples as well as a control sample were prepared in vitro. In addition to substrates, ions of potassium, sodium and zinc were added to each sample and then, acid phosphatase activity of each sample was read by spectrophotometer in the determined wavelength.

Results and Conclusions: Zinc, sodium and potassium ions can increase activity of acid phosphatase up to 87.76%, 63.53% and 18.12%, respectively. The results of this study revealed that all three ions of zinc, sodium and potassium can increase acid phosphatase activity.

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Introduction

Acid Phosphatase enzyme, named Prostatic Acid Phosphatase, belongs to the family of hydrolyses, that there are several isoenzymes with common enzyme function. This enzyme is a protein formed from several polypeptide chains and its spatial structure is formed in such a way that after the stand of substrate (Paranitrophenyl phosphate) in their active sites, relevant reactions are activated. Especially, acid phosphatase targeted phosphate groups and breaks them [1]. Acid phosphatases are enzymes widely distributed in tissues, including the bone, liver, spleen, kidney, red blood cells, and platelets. However, their greatest diagnostic importance involves the prostate gland, where acid phosphatase activity is 100 times higher than other tissues [2]. Elevated levels of acid phosphatase are observed when prostate cancer has metastasized beyond the capsule to the other parts of the body, specifically the bone. Once the carcinoma has spread, the prostate starts to release acid phosphatase, resulting in an increased blood level. Acid phosphatase is also present in high concentrations in the seminal fluid. Tests for presence of this enzyme may be used on vaginal swabs to investigate the rape [1, 2].

Reduced level of acid phosphatase may occur after estrogen therapy in patients with prostate cancer. After successful treatment of estrogen, it may take several weeks to return to normal levels of acid phosphatase. Just a few days after the successful treatment of prostate tumor by surgery, acid phosphatase level will decrease. In patients with Down syndrome, reduced levels of acid phosphatase may be demonstrated. In some cases, decreased presence of cofactors may also reduce acid phosphatase activity [2]. Phosphatase enzymes are the most important plant enzymes that playing a role in intracellular and extracellular. As a matter of fact, their roles involve dephosphorylating of inorganic phosphate and its conversion to organic phosphate. Phosphatases, are divided to acid phosphatase and alkaline phosphatase depending on the optimal PH [1-3].

The enzyme acid phosphatase provides phosphate to tissues that have high energy needs during growth and maturity [4]. This enzyme is found in five major forms in the body: Prostate, lysosomes, erythrocytes, macrophages and bone [5]. These enzymes are found in erythrocytes, leukocytes, spleen, platelets, kidneys, bones and other tissues [1].

Enzymes can generally be divided into two categories. Activity of a number of the enzymes depends only on their protein structures; whereas some other enzymes need non-protein active components (cofactors) for their catalytic activity.

Cofactors consist of two types: A) Cofactors involving ion Metals Na, Cu, Mg, Mn, Fe, Zn and K. These cofactors can be divided into two sorts as well:

1. Ion metals are responsible as an interface for enzyme and substrate of molecules.
2. Some of the metal ions self-participate in catalysis reactions, including iron atom in the catalase enzyme which has a main role in the

decomposition of hydrogen proxy. B) Cofactors, composed of an organic molecule, which are named coenzymes [1, 4, 5].

Given the importance of enzymes and their level changes that occur in certain diseases and cofactors and inhibitory role of ions in the increase or decrease its level in those cases that it can be considered as a treatment for this disturbances, this study was done.

Materials and Methods

Acid phosphatese enzyme isolation from orange

At first, 50 g of orange were washed by tap water and finally with distilled water, and then normal saline was added four times of its weight, which was kept in the refrigerator for 16 hours. Then the mixture was mixed in cold conditions, to which the saline was added in two stages and in each stage, after stirring in the cold conditions for 3-4 hours, centrifuges at 80000 RPM for 10 minutes. Then at the end stage of the second phase, the obtained surface layer and the sediment from the two phases were mixed together and after the measurement, equal volume of cold Butanol drops was added in cold conditions for 4 hours and while stirring was added. Prepared homogeneous mixture was kept in the refrigerator and after second stirring it was centrifuged in the cold conditions. In this stage, the surface layer were discarded, containing Butanol, pigments, and fats, and bottom layers containing the enzyme were collected. In the next stage, 30-70 volume ratio of cool acetone were added to the mixture containing enzyme, in totally cool conditions. The obtained contents from Buchner funnel was finally passed and then, the remaining white precipitate was kept and dried (this precipitated white powder is named precipitated powder with acetone). Then, to 40 grams of precipitated powder with acetone, about 22 times of their weight, normal saline was added in two stages and at any stage, the stirring and then centrifugation was done in cool conditions. At the end stage of the second phase, the sediment layer was discarded and the surface layer was kept in the refrigerator. The obtained top layer was precipitated in the two phases, first by 30% and then with 80% ammonium sulfate. Precipitation via applying 30% ammonium sulfate was discarded, but precipitation from 80% ammonium sulfate was maintained. At last, , ammonium sulfate in the sediment were removed out utilizing a dialysis bag and after the $BaCl_2$ test, the content of the dialysis bags were centrifuged to ensure the complete withdrawal of ammonium sulfate. The precipitation layer was discarded and the supernatant layer was held. The kept surface layer contained the enzyme [6].

Measurement of the enzyme

Several concentrations were prepared from each compound and for each concentration, a test tube and for all concentrations, a control was considered. In each test tube, 2 ml of normal saline, 1 ml of buffer with pH 5.2, 0.2 ml of samples containing enzyme and 0.2 ml of inhibitor were added and all the tubes were placed in the laboratory conditions for 5 hours. Then, 0.2 ml of substrate with 1 millimolar concentration was added to all tubes after 2 hours, 1 ml of NaOH 10% was added and their

absorbance was read at wavelength of 405 nm. This experiment was repeated three times to ensure the results [6].

In each of K_2SO4 solution, NaF and $ZnSO_4$ concentrations of 1, 2, 4, 8, 16 and 20 micromolar were prepared and acid phosphatase activity was measured against various concentrations of each of these ions.

Results and Discussion

On samples obtained from the two stages of the extraction and purification of acid phosphatase (Precipitation after use of acetone and Precipitation with dialyzed ammonium sulphate 80%), acid phosphatase activity, protein amount and specific activity of the samples were measured (Table 1).

Table 1. Measurement of protein amount in two stages of enzyme extraction

	Acid phosphatase activity	Protein (mg/dl)	Specific activity (U/mg)
Precipitation after use of acetone	6.6	12.2	0.54
Precipitation with dialyzed ammonium sulphate 80%	4.5	11.5	0.39

In the acid phosphatase extraction process, a decline in the amount of protein was observed from the first stage to the next stage. Acid phosphatase activity was measured in presence of 1, 2, 4, 8, 16 and 20 micromolar concentrations of K_2SO4, NaF and $ZnSO_4$.

All three metal ions of potassium, sodium and zinc increased acid phosphatase activity on ammonium fraction of acid phosphatase of orange fruit. In concentrations of 1 to 8 micromolar, activity was increased and in higher concentrations, activity remained steady. Most effects were reported in regard with 8 micromolar concentration (Table 2).

Table 2. Percent of acid phosphatase activity in the presence of different concentrations of potassium, sodium and zinc ions

	1	2	4	8	16	20
K2SO4	4.25	9.36	14.72	18.12	18.20	18.19
NaF	17.65	31.82	49.30	63.53	63.57	63.60
ZnSO4	21.18	34.69	59.33	87.76	87.83	87.88

Zinc ions significantly increased activity of the acid phosphatase enzyme up to 87.76% (p=0.000). Moreover, sodium and potassium ions increase activity of the acid phosphatase enzyme to 63.53% (p=0.004) and 18.12% (p=0.01), respectively. Stimulatory effect of Potassium, Sodium and Zinc metal ions were demonstrated to be significant on activity of acid Phosphatase on ammonium fraction of acid Phosphatase of Orange fruit.

Acid phosphatase enzyme belongs to the hydrolysis family that prepared phosphate for tissues demanding a high energy during the development and maturation. This enzyme is located in the Lysosomes and actions through hydrolysis of acid phosphoric monoesters as well as its conversion to phosphate ion. A molecule with one free hydroxyl group makes apart phosphate group from their precursor. This enzyme in the human body seems to be effective on the ovary's metabolic activity such as egg maturation, restart of mitosis division, breakage of germinal vesicles and ovulation. Furthermore, with autophagia and heterophagia, digest yellow body and atresia follicle. This enzyme is controlled by steroid hormones [4, 7].

Elevated levels of acid phosphatase can be observed in regard with prostatic carcinoma, benign prostatic hypertrophy, prostatitis, multiple myeloma, paget disease, hyperparathyroidism, metastasis to the bone, sickle cell crisis, thrombocytosis, lysosomal disorders (e.g., Gaucher disease), renal diseases, liver diseases (e.g., cirrhosis) and rape [2, 8]. Reducing the level of acid phosphatase may occur after estrogen therapy in patients with prostate cancer. After successful treatment of estrogen, it may take several weeks to return to normal levels of acid phosphatase. Just a few days after the successful treatment of prostate tumor by surgery, acidphosphatase level will decrease. In patients with Down syndrome, reduced levels of acid phosphatase may also be observed. In some cases، decreased presence of cofactors may also reduce acid phosphatase activity [2].

This enzyme is a key enzyme in the soil phosphorus cycle and that activity is an indicator to determine the mineral potential and soil biological activities [9, 10]. Some enzymes activities require the presence of cofactors, by which their activity increases. Normal activities of enzymes seem to be essential for normal functions and health. Acid phosphatase enzyme is involved in the metabolism regulation of calcium, phosphorus and sugar by phosphorylation/ dephosphorylation mechanism. Obviously, disrupting the level or activity of these enzymes, can cause disorders in bone [11, 12]. Therefore, applying this cofactor, necessary for normal activity of acid phosphatase enzyme, may be beneficial to treatment of cases which reduce activity of this enzyme.

Conclusion

The findings of this study demonstrated that zinc, sodium and potassium ions can increase acid phosphatase activity that the higher activity belonged to zinc ion. Utilizing these cofactors, may be helpful in increasing acid phosphatase activity and may treatment useful in diseases that outcome of decreased acid phosphatase activity and in cases with decreased acid phosphatase activity in future.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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