Часткове очищення каталаза від огірка (*cucimus sativus*)

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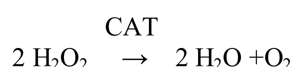
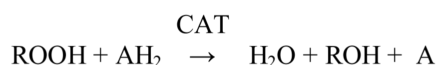
Каталаз (CAT) (EC 1.11.1.6) – це тетрамерний фермент, що складається з чотирьох ідентичних тетрагедрально розміщних субодиниць вагою 60 кілодальтонів, що містить одну феріпротопропінову групу на субодиницю, і має молекулярну масу близько 240 кілодальтонів [1,2]. CAT дуже ефективно реагує з H_2O_2, формуючи воду та молекулярний кисень; та з H –донорами (метанол, етанол, мурашина кислота , або феноли) з пероксидазною активністю :



$$2\,H_2O_2 \xrightarrow{CAT} 2\,H_2O + O_2$$



$$ROOH + AH_2 \xrightarrow{CAT} H_2O + ROH + A$$

У тварин, пероксид гідрогену детоксифікується з допомогою CAT та GPX. Каталаз захищає клітини від пероксиду водню, який виробляється в них. Хоча CAT не достатній для певних типів клітин за нормальних умов, він відіграє важливу роль у виробленні стійкості до оксидативного стресу у адаптаційній реакції клітин. У цій дослідницькій роботі аткивність каталазу (E.C.1.11.1.6) визначалася у клітковині огірка (Cucimus Sativus) активність було визначено після гомогенізації, ультрацентрифугізації та DEAE-целюлозного іонного обміну та вертикальної хроматографії. Після ультрацентрифугізації, активність каталазу було встановлено як 46.51 Од/мг прот. та 3.19 Од/мг прот. відповідно. Після демінералізації з допомогою вертикальної хроматографії, спеціальну активність каталазу було встановлено так: 393 Од/мг прот. та 0.479 Од/мг прот. відповідно. Ферментні препарати зберігалися при температурі через 96 годин каталаз втратив майже всю свою активність.

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Partial purıfication of catalase from the cucumber (*cucimus sativus*)

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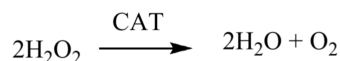
In this study, catalase (E.C.1.11.1.6) activities were determined in the extract of cucumber (Cucimus Sativus) activities were determined after homogenization, ultracentrifugation and DEAE-cellulose ion exchange column chromotography steps After the ultracentrifugation step, catalase activities were found as 46.51 U/mg prot. and 3.19 U/mg prot., respectively. Afterdesalting with column chromotography, catalase spesific activities were found as 393 U/mg prot. and 0.479 U/mg prot., respectively. Enzyme preparations were stored at after 96 hours catalase lost almost all of its activity.

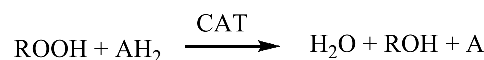
Keywords – catalase, cucumber, purification, chromatography.

I. Introduction

Catalase (CAT) (EC 1.11.1.6) is a tetrameric enzyme consisting of four identical tetrahedrally arranged subunits of 60 kDa that contains a single ferriprotoporphyrin group per subunit, and has a molecular mass of about 240 kDa[1,2]. CAT reacts very efficiently with H_2O_2 to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, or phenols) with peroxidase activity



$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$



$$ROOH + AH_2 \xrightarrow{CAT} H_2O + ROH + A$$

In animals, hydrogen peroxide is detoxified by CAT and by GPX. Catalase protects cells from hydrogen peroxide generated within them. Eventhough CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells.

In this study, catalase (E.C.1.11.1.6) activities were determined in the extract of cucumber (Cucimus Sativus) after homogenization, ultracentrifugation and DEAE-cellulose ion exchange column chromotography steps After the ultracentrifugation step, catalase activities were found as 46.51 U/mg prot. and 3.19 U/mg prot., respectively. Afterdesalting with column chromotography, catalase spesific activities were found as 393 U/mg prot. and 0.479 U/mg prot., respectively. Enzyme preparations were stored at after 96 hours catalase lost almost all of its activity.

In this study, cucumber were obtained from the Cukurova University, Faculty of Agriculture, Department of Horticulture. 10 g cucumbers were homogenized containing 50 mM 0.5% Polyviniylpolypyrolodine (PVP)

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pH:7.0 were homogenized in 25 ml of K2HPO4. The resulting homogenate was filtered and centrifuged at 12.000 x g for 30 min were stored at 4°C [3,4]. Catalase in the cucumber homogenates, % 0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100 respectively, were precipate of the ammonium sulfate solution. The ammonium sulfate fraction was applied onto a DEAE-cellulose column previously equilibrated with the phosphate buffer (pH:7.0). Elution flow rate was choosen as 3 ml/min. Catalase activity determined according to the Aebi et al.[5]

Catalase activity was 26.56 U/mg in crude homogenate. Protein concentration were determined according to the Lowry et al. [6] and concentration found as 3.99 mg/ml.After ammonium sulfate precipitation, the amount of protein 3.19 mg / ml, catalase, specific activity value was found 46.51 U / mg . Then the application of DEAE-cellulose ion exchange column chromatography specific catalase activity was found as 393 U/mg. Purification of catalase in cucumber was 1.75 times with centrifugation, DEAE-cellulose ion exchange column chromatography 14.7 times with comparing crude homogenate. Catalase activity were measured from 5.0 to 8.0 and the optimum pH was determined as 6.6. Catalase activity were measured from 0 to 90 °C and the optimum temperature was determined as 32 °C. Storage stability of enzyme was determined at 4 °C .All enzyme activity was lost after 96 h. Michealis_Menten kinetics were determined for purified catalase. V_{max} and K_m were found as 76.92 U/mg protein and 0.01796 Mm.

Conclusion

DEAE-cellulose ion exchange column chromatography specific catalase activity was found as 393 U/mg. Purification of catalase in cucumber was 1.75 times with centrifugation, DEAE-cellulose ion exchange column chromatography 14.7 times with comparing crude homogenate.

References

[1] P.T. Vasudevan, R.H. Weiland, " Studies on the Morphology of Immobilized Catalase", The Chemical Engineering Journal, 55:B41-B45, 1994.

[2] B. Hallıwel, J.M., Gutterıdge, Methods Enzymology, 186:185. 1990.

[3] J.M. Mates, C.Perez-Gomez,I.N. Castro, "Antioxidant Enzymes and Human Diseases", Clinical Biochemistry, 32: 8, 595–603, 1999.

[4] E. A. Havir, N. A. Mchale, Plant Physiol,84:450-455, 1987.

[5] E.A. Havir, N. A. Mchale, "Purification and Characterization of an isozyme Catalase with Enhanced-Peroxidatic Activity from Leaves of Nicotiana Sylvestris", Archivesof Biochemistry and Biophysics, 283:491-495. 1987.

[6] H. Aebi, S.R. Wyss, B. Scherz, F. Skvaril, "Heterogeneity ofErythrocyte Catalase. II. Isolation and Characterization of Normal and Variant Erythrocyte Catalase and Their Subunits", Eur J. Biochem, 48: 137, 1974.

[7] O.H. Lowry N.J. Rosebrough , A.L. Farr et al. "Protein measurement with folin phenol reagent", J Biol Chem, 193:261, 1951

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