THE INFLUENCE OF SOME CATIONS OVER THE ALCOHOL DEHYDROGENASE ACTIVITY

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Abstract

It was studied the influence of the cations Ca^{2+}, K+, Mn^{2+}, Ni^{2+}, Co^{2+}, Pb^{2+}, Fe^{3+}, Sn^{2+}, Hg^{2+}, over alcohol dehydrogenase activity using UV spectrophotometer kinetic method.
Key words: cations, alcohol dehydrogenase, spectrophotometric

INTRODUCTION

Alcohol dehydrogenase (EC 1.1.1.1.) catalyzes the reversible reaction of ethanol oxidation to acetaldehyde in the presence of redox system coenzymes NAD^+/NADH + H^+, by the reaction:

$$R - CH_2 - OH + NAD^+ \leftrightarrow R - CH{=}O + NADH + H^+$$

The enzyme that has been isolated from yeast has a molecular weight of 141000, an isoelectric pH of 5.4 and is a tetramer. On each subunit there is an atom of zinc and two thiol groups that are essential for catalytic process [1].

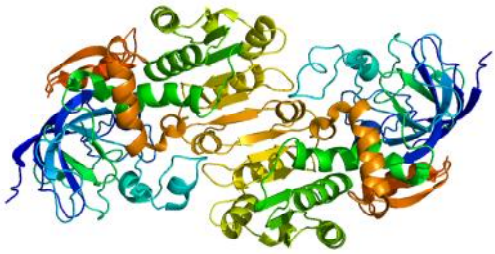

Fig. 1. Alcohol dehydrogenase [1]

Alcohol dehydrogenase activity is related to the ethanol concentration in wine fermentation.

MATERIALS AND METHODS

The reaction rate of the alcohol dehydrogenase is determined by monitoring the increase in the extinction at 340 nm of the reaction mixture, as determined by a corresponding reduction of NAD^+.
An enzyme unit corresponds to the reduction of one micromole of NAD^+ per minute at 25° C under the defined conditions [3].

Reagents

1. Buffer sodium pyrophosphate 50 mM, pH 8.82
2. Ethanol 2M
3. 15 mM NAD^+ aqueous solution
4. 10 mM sodium phosphate buffer
5. Bovine Serum Albumin 500 mg in 500 NAD^+
6. Alcohol dehydrogenase (0.1 mg/ml in buffer 4) isolated from Aspergillus niger
7. Sodium phosphate buffer solution + human serum albumin (0.1%)

All reagents were purchased from Sigma Aldrich, Germany.

In two spectrophotometer cuvettes are introduced the reagent as presented in *Table 1*.

Table 1. Work mode

Reagents	**Sample**	**Control**
Buffer sodium pyrophosphate (ml)	0,6	0,6
Ethanol (ml)	0,6	0,6
NAD^+ (ml)	0,7	0,7
Alc-DHG (ml)	0,1	-
Buffer solution (ml)	-	0,1

The optical density of the sample with enzyme is determined at 340 nm and compared to a blank at time zero and in 10 seconds intervals for 10 minutes.

Results calculation

The change in optical density at 340 nm is plotted versus time on the initial linear portion of the curve.

$$Units \cdot ml = \frac{D.O._{340} \cdot min}{6,2}$$

where,
$6,2 \times 10^6$ cm^2/ml is the extinction coefficient of NADH at 340 nm.

The specific enzyme activity is expressed in units/mg of protein and take into account the dilution factor applied.

RESULTS AND DISCUSSIONS

It was studied the influence of some cations Ca^{2+}, K+, Mn^{2+}, Ni^{2+}, Co^{2+}, Pb^{2+}, Fe^{3+}, Sn^{2+}, Hg^{2+}, over alcohol dehidrogenase activity using spectrophotometer method (Spectrophotometer Specord 210 Plus UV-VIS Analytic Jena, software Win ASPECT PLUS) after the work mode presented in *Table 2*.
The results are presented in figures 2 and 3.

Table 2. Work mode 2

Reagents	Test	Control
pyrophosphate buffer (ml)	1,5	1,6
Ethanol (ml)	0,5	0,5
NAD^+ (ml)	1	1
ALC-DHG (ml)	0,1	-
Cations* (ml)	0,1	0,1

*As cations were used aquas solution of $CaCl_2$, KCl, $FeCl_3$, $MnCl_2$, $HgCl_2$, $SnCl_2$, $CoCl_2$, $NiCl_2$, $PbCl_2$ in concentration of 0,1 M.

The experimental results are presented in figure 2.

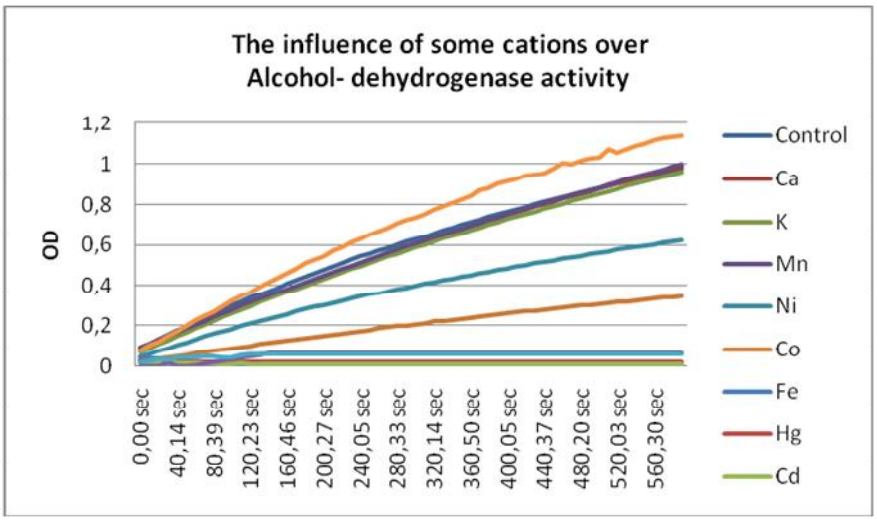

Fig. 2. The influence of some cations over alchohol dehydrogenase activity

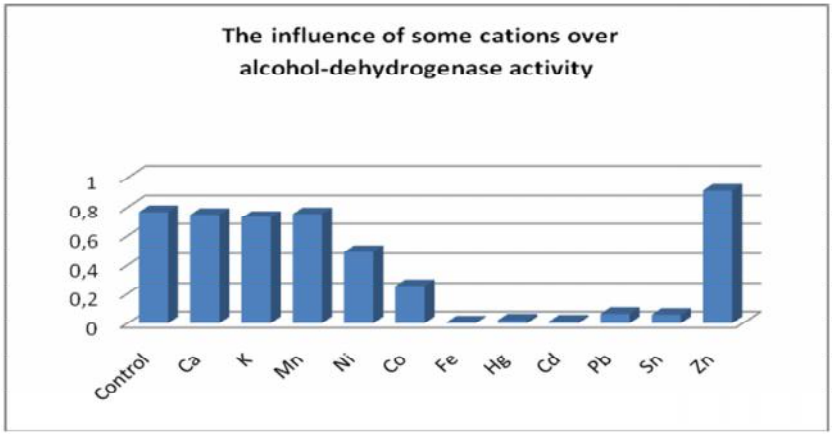

Fig. 3. The influence of some cations over alchohol dehydrogenase activity

CONCLUSIONS

It is notice that the cations are influenced the alchohol dehydrogenase activity in a different manner:
-Hg and Mn ions are denaturated the enzyme;
- Pb and Fe ions are strong inhibitors for alchohol dehidrogenase activity;
- Co, Ni and Sn are moderate inhibitors for alchohol dehidrogenase activity;
- Ca and K ions are activators for alchohol dehidrogenase activity

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