

## Research Article

# The effect of amino acid salts on activity and stability of alcohol dehydrogenase

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## **ABSTRACT**

**Background:** In the high concentration of salts, kosmotropic and chaotropic effects became clear and these effects are related with Viscosity B-coefficient.

**Methods:** In present study, the effects of high concentration of amino acid salts (1-3M) on yeast alcohol dehydrogenase was assessed and the effects of Na.Aspartate, Na.Glutamate, HCl.Glycinate, HCl.Lysinate and HCl.Lysinate on stability and activity of the enzyme was evaluated.

**Results:** The rate of reduction of enzyme activity is reduce in presents of salts with kosmotropic anions and the half-life of the enzyme is greater in presents of Na.Aspartate and Na.Glutamate that have positive Viscosity B-coefficient. As well, there is a linear relation between activity and stability of enzyme and dB.

**Conclusion:** It can be expressed that kosmotropes and chaotropes have different effects on stability and activity of enzyme and both activity and stability of enzyme are related with kosmotropic and chaotropic effects.

**Keywords:** Yeast Alcohol Dehydrogenase, Amino Acids, Chaotrope, Kosmotrope, Enzyme Kinetic

## **INTRODUCTION**

Oxidoreductase enzymes are enzymes that catalyze different types of redox reactions, and often are dependent on the coenzymes such as nicotinic amide adenine nucleotide ( $\text{NAD}^+$ ) and nicotinic amide adenine dinucleotide phosphate ( $\text{NADP}^+$ ). Dehydrogenases are important groups of oxidoreductase enzymes. Alcohol dehydrogenase is typical of this group. Alcohol dehydrogenase is a metalloenzyme containing Zn which catalyzes the conversion of ethanol and  $\text{NAD}^+$  to acetaldehyde and NADH in a bilateral reaction (Plapp et al., 1993). Yeast alcohol dehydrogenase (YADH) (EC.1.1.1.1) with a molecular weight of 150 kDa is a homo-tetramer. All of its four subunits are the same each containing 347 amino acid. There are two zinc

atoms per subunit, so it is a metalloenzyme (Veillon et al, 1975). One of the metal ions of zinc in each subunit has a structural role and protects enzyme conformation. The other ion participates in catalysis process, and is called catalytic zinc. The structural zinc plays an important role in conformation stability against denaturation due to temperature changes (Leskovac et al., 2002).

Natural conformation stability of biological macromolecules is closely associated with non-covalent bonding such as hydrogen bonds, electrostatic and hydrophobic interactions. These interactions are affected by solute and solvent that has surrounded macromolecules. Due to complex conformational structures and three-dimensional structures of protein, studying the direct effect of

solvent/ solute on biological macromolecules is extremely challenging. In other words, changing behavior of model compounds such as amino acids and peptides will be helpful to understand the water-protein interactions in soluble. Most of amino acids and their derivatives are known as compensatory and compatible soluble in stabilizing protein and increasing their activities. Since amino acids are zwitterions in aqueous solutions, their hydration and interaction with proteins have many similarities with electrolytes (Chen et al., 2010). Considering that amino acids have unique zwitterions structure, their viscosity coefficients B provide the opportunity to compare the ability of amino acids in stabilizing proteins. Adding osmolites to reaction is one of the most important methods in enzyme stability studies. In this connection, after salts are in solvent they are isolated as constructive ions. Therefore, ions are divided into two categories:

Kosmotropic anions stabilize protein and cause protein precipitation. Chaotropic anions make proteins unstable (Chen et al., 2010). Strongly hydrated ions are known as kosmotropes or structure-maker, and those ions that are weakly hydrated are known as chaotropes or structure-breakers (Marcus., 1994; Collins., 1995, M. Vanderkooi). Viscosity coefficient B, which describes ion- solvent interactions, is interpreted as a measure of structure-making or structure-breaking capacity of an ion in solvent, thus it is directly related to kosmotropic/ chaotropic properties. Viscosity coefficient B is usually (+) for kosmotropes and is (-) for chaotropes. The effects of kosmotropic-chaotropic are most evident when electrostatic forces are eliminated and ion diffusion forces become dominant (S.Lawal, 2006, Yang, 2009 B. Jenkins, 1995). In addition to the effects of ions on protein activity, ions influence protein stability that is again related to viscosity coefficient B. Since in high concentrations of salt, some forces become obvious and some other are eliminated (Baudium, 2004), therefore, in this study, the high concentration of amino acid salts and their effects

on the activity and stability of yeast alcohol dehydrogenase were used.

## 2-MATERIALS AND METHODS

**2.1. Material:** Yeast enzyme alcohol dehydrogenase (YADH), ethanol and  $\text{NAD}^+$  Sigma were purchased. Sodium glutamate amino acids, aspartate glutamate, glycine chloride, arginine chloride, and lysine chloride were purchased from Fisher, Aldrich and Merck.

### 2.2. Preparation of enzyme solutions, buffer, substrate, buffer solution/ salt

#### 2.2.1. Preparation of enzyme solution:

An enzyme solution with the dissolution of 1 mg in 1 ml of phosphate buffer solution was prepared. For enzyme studies, diluted enzyme solution should be used, thus enzyme was diluted 40 times.

#### 2.2.2. Preparation of sodium phosphate buffer (50 mM, pH 7.8):

Deionization of sodium phosphate's buffer was carried out using two salts of monobasic and dibasic sodium phosphate and adding certain amount of these salts in water. Morality and pH were adjusted with the above mentioned salts. Enzyme, amino acid salts and ethanol were solved in this buffer.

#### 2.2.3. Preparation of Tris buffer (5mM, pH 7)

To prepare coenzyme solution, according to morality, a certain amount of salt Tris-HCl is weighted for certain volume. It is reached to the intended concentration using deionized water and buffer pH was adjusted on 7 by diluted NaOH or HCl.

#### 2.2.4. Preparation of different substrate solutions

To determine the kinetic parameters and enzyme stability studies, Ethanol that is enzyme substrate was prepared in different concentrations (ten concentration) and a concentration of substrate, with maximum speed, was used for stability studies and determining half-life. It should be noted that to determine enzyme activity in the presence of various salts of amino acids, the substrate was dissolved in salty solutions.

### 2.2.5. Preparation of the amino acid salt solutions

Buffer-salt solutions were prepared by dissolving different amino acid salts in sodium phosphate buffer (50mM and pH 8/7). To prevent the reduction in enzyme activity, after the dissolution of salts, using NaOH or HCl, pH was again adjusted on 8.7. the concentrations of 1,2,3 M Sodium glutamate, aspartate glutamate, glycine chloride, arginine chloride, and lysine chloride were prepared in accordance with molarity equation.

### 2.3. Enzyme activity measurement

Enzyme activity was determined after the formation of NADH at 25°C. Following the addition of 10 µL enzyme solution cuvette containing 980 µL ethanol (dissolved in sodium phosphate buffer) and 10 µL NAD<sup>+</sup>, the solution was stirred gently. The increasing of absorption in 340 nm was read via spectrophotometer. To study the effect of amino acid salts, ethanol was dissolved in prepared concentrations of each salt. The initial velocity (molL<sup>-1</sup> S<sup>-1</sup>) of enzymatic reaction was obtained from the ratio of OD changes by time changes. At the first minutes of experiment, reaction was linear. To determine enzyme activity, test was performed three times and reaction velocity of samples was measured at 25 °C to control the accuracy of results. Michaelis–Menten equation was used to determine kinetic parameters. Two substrate reaction of yeast alcohol dehydrogenase follows completely Michaelis–Menten model. Using Excell software and extrapolation of points on Michaelis–Menten graph, kinetic parameters were obtained. By definition, a unit of alcohol dehydrogenase enzyme is a dose that converts 1 µmols NAD<sup>+</sup> to NADH<sup>+</sup> at 25 °C.

### 2.4. Enzyme stability studies

For yeast enzyme, a diluted enzyme solution in buffer and diluted in each of above mentioned amino acid salts in hot water bath were incubated in 40°C and 50 °C. Consequently, 10 µL of each enzyme solution was taken to measure enzyme activity at 25 °C. Time-dependent loss of activity

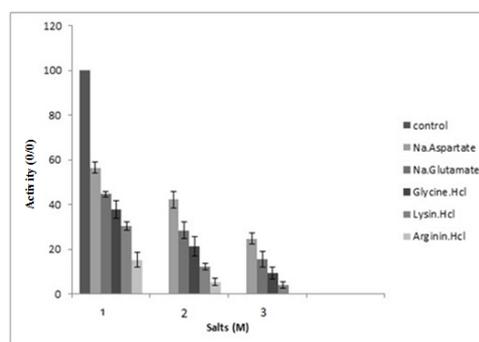
was used to calculate the half-life of each enzyme solutions, and this is the equivalent of enzyme stability kinetics.

### 2.5. Statistical analysis of Data

The results of this study were analyzed using SPSS software Version 18 and One-way annova method and the results were significant at the level of P <0.05.

### 3. Results

#### 3.2. Obtained results from the effect of amino acid salts on enzyme activity



**Figure 1-** The effects of various concentration of amino acids salts on activity of enzyme

#### 3.3. Obtained results from the effect of amino acid salts on enzymes stability:

Table-1 and 2 show the effects of amino acid salts on enzymes stability in 40°C and 50°C respectively

**Table-1.** Half-life of enzyme in presence of amino acid salts in 40°C

Salts	Half-Life (min)		
	1M	2M	3M
control			48
Na.Aspartate	1290	960	450
Na.Glutamate	320	14	55
Glycinate.HCl	175	85	27
Lysine.HCl	70	25	10
Arginine.HCl	36	25	9

**Table -2.** The half-life of enzyme in the presence of amino acids salts at 50°C

Salts	Half-Life (min)-		
	1M	2M	3M
control			35
Na.Aspartate	180	95	45
Na.Glutamate	85	68	20
Glycinate.HCl	60	38	12
Lysine.HCl	30	12	0
Arginine.HCl	10	3	0

### 3.4. Obtained results from the effect of amino acid salts on enzyme kinetic parameters

**Table-3.** The effect of viscosity coefficients of amino acids salts, their ions on enzyme kinetic parameters.

Salt	B <sup>-1</sup>	B <sup>+1</sup>	B <sup>-</sup> B <sup>+</sup>	dB/dT	K <sub>M</sub> (mM)	V <sub>max</sub> (μM/min)
Control				-	32	0.19
Na.Aspartate	0.13	0.085	0/045	4	29	0.13
Na.Glutamate	0.29	0.085	0.205	2.6	27	0.11
Glycinate.HCl	-0.005	0.143	0.138	1.4	43	0.09
Lysine.HCl	-0.005	-	-	-	47	0.085
Arginine.HCl	-0.005	0.5013	0.4963	-2.6	48	0.21

## 4. DISCUSSION

### 4.1. The effect of amino acid salts on enzyme activity

The studied amino acid salts decrease enzyme activity (Figure1). Salts change the enzyme activity by affecting its kinetic parameters, especially K<sub>m</sub>. If salts react desirably with substrate, its tendency to enzyme will be decreased, and consequently K<sub>m</sub> will be increased. It is worth mentioning that since both cations and anions of salts may interact with enzyme and affect its active sites; therefore, they can change enzyme K<sub>m</sub> and V<sub>max</sub>, too (Table 3). The alcohol dehydrogenase enzyme contains catalytic and conformational Zn (alcohol dehydrogenase has four Zn, two are catalytic and the other two are conformational, which are involved in enzyme structural integrity) (Duester et al., 1999). Zn ions available in the active site of enzyme are strongly kosmotropic cations that have more tendency to connect with strong kosmotropic anions (Collins, 1997). Zn of enzyme active site interacts with kosmotropic anions such as aspartate and glutamate and reduces enzyme activity. In particular, the strong interactions between this metal ion and kosmotropic anions may lead to a reduction in Nucleophile of hydroxyl ion coordinated with Zn (Chen et al., 2010). It can be concluded that the effect of salts on the enzyme structure is the most important factor in inhibiting enzyme activity. In addition, since it does not seem the change in the fourth structure affect enzyme activity (M.Bowers et al., 2007), this effect may be due to a change in the second and third structure of enzyme. Reduction in enzyme activity in the

presence of chloride arginine is due to reduction of enzyme's substrate fraction that exists in enzymes active site. It is expected that increasing in salt concentration decreases pK<sub>a</sub> of COOH. Therefore, amino acid salt may play a similar role as organic solvent in affecting on enzyme function since it removes the essential water of enzyme and penetrates into liquid microphase in order to interact with enzyme by changing protein dynamics, protein conformation or by penetrating into the enzyme active site, amino acid salts either interact directly with substrates and products or interacts indirectly with substrates and products by changing the distance between the aqueous and non-aqueous phases (Yang, 2009). Chaotropic anions decrease superficial K<sub>cat</sub>, but increase appearance K<sub>m</sub>, while kosmotropic anions decrease both appearance K<sub>cat</sub> and enzyme K<sub>m</sub> (M. Bower, 2007) (Table 1).

### 4.2. The effect of salt on enzyme stability

In this study, enzyme stability was investigated both in 40 and 50 °C. In these high temperatures, different salts show different stabilizing effects (Collins, 1997). The half-life of enzyme increases gradually only by increasing B<sup>-</sup> or (B<sup>-</sup>-B<sup>+</sup>) of added salt (Tables 1 and 2). In the presence of salts with kosmotropic anions, enzyme keeps its structural integrity while chaotropic anion causes enzyme dissolution and disturbance of enzyme natural conformation (Kunz et al., 2004). Kosmotropic anions don't have tendency to interact with protein, while chaotropes remove essential water of enzyme and interact with chaotropic cation groups of enzyme (like amino groups) and amid groups in peptide backbone, which are buried in protein in normal situations. This leads to enzyme denaturation (Barreca et al.,

2009). Kosmotropic anion are paired with chaotropic cation, this reduces the stabilizing effect of Kosmotropes. There is a relationship between the half-lives of enzyme and salt viscosity B-coefficients. When anion viscosity coefficient is positive, enzyme half-life is high. High concentrations of salt allow conformational changes in protein that cause a reduction in the interaction between catalytic residues (Bonnete et al., 1999). When enzyme is exposed to high temperature, it maintains its activity with amino acid salts as the following:

<< Na.Glutamate < Na.Aspartate.Glysinate.HCl

Arginin.HCl<Lysine.HCl

It means that the lowest enzyme activity occurs in the presence of arginine chloride ,and the highest enzyme activity occurs in the presence of sodium aspartate. Chaotropes break hydrogen bond network of water and thus allow macromolecules to stimulate protein toward denaturation by more structural freedom while kosmotropes are structure-maker, reduce surface tension and increase the stability of macromolecules structure (Hofmeister et al., 1888). Anions compared to cations have a more dominant role in impacting the stability of enzymes (Galinski et al., 1997).

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