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Biochemical characterization of lysozyme extracted from Caspian kutum, *Rutilus kutum*, Kamensky 1901

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ABSTRACT

This paper presents the findings of a basic study on biochemical characteristics and activity of lysozyme extracted from a commercially important fish, Caspian kutum, *Rutilus kutum*, Kamensky 1901. The enzyme was purified by the ammonium sulphate precipitation method and cationic exchange chromatography. Molecular weight of lysozyme was estimated as 15.8kDa by SDS-PAGE. The optimum pH and temperature were 6.0 and 45.0°C, respectively,while the K_m and V_{max} values were 0.00007g mL^{-1} and 200units min^{-1}, respectively. Thermo-stability of the enzyme was low at the temperatures higher than 50°C. The enzyme activity increased in the presence of NaCl (1-50mM), KCl (1-50mM) and $CrCl_2$ (1-5mM), then decreased at the higher concentrations. Furthermore, the enzyme activity significantly decreased in the presence of $FeCl_2$ and $CuCl_2$ (1-100mM), but not stable in the presence of $MgCl_2$. SDS inhibited the activity, but the enzyme exhibited a good degree of resistance to urea. Fluorescence quenching was observed in the presence of $FeCl_2$ and $CuCl_2$. Emission intensities in the presence of NaCl, KCl, $CrCl_2$ and SDS were elevated, while was negligible for the urea. Our results exhibitedthat NaCl and KCl are well-established salts for elevating the enzyme activity.However, the purified lysozyme did not display a moderate stability against others salts. Besides, the purified lysozyme from *R. kutum* was not heat stable. Therefore, some phenomena such as water hardness and warming can exert negative effects on the innate immunity of the fish. So, the enzyme structure can be made more stable by medium engineering through changing the salt composition of aqueous solution.

Key words: Characterization, Lysozyme, *Rutilus kutum.*

INTRODUCTION

Lysozymes (EC 3.2.1.17) belong to the group of alkaline glycosidases which generally are known as β-1, 4-N-acetyl muramidase. These enzymes hydrolyze the β 1,4 glycosidic linkage between N-acetylmuramic acid and N-acetylglucosamine of peptidoglycan in the bacterial cell wall. Various types of these enzymes have been identified: chicken-type (c-type), goose-type (g-type), plant-type, invertebrate-type (i-type), phage-type. There are three types of these enzymes in animal kingdom: c-type, g-type, and i-type (Hikima et al. 2001). In primitive vertebrates, innate immune system plays critical role against infection diseases. The innate immune system of fishes has more differences or limitations than higher vertebrates, especially in antibody–based immunity. Antibodies and the very limited number of immunoglobulin isotypes (1 or 2) are found in fishes compared to higher vertebrates (Lie *et al*. 1989). The lysozymes as key molecules in innate immunity are considered as one of the important anti-bacterial molecules to fight against pathogens in fish (Uribe *et al*. 2011).

Over the past decade, the c-type and g-type lysozymes have repeatedly been reported in the tissues of head kidney, skin, gastrointestinal tract, gill and eggs, which are at a greater risk for bacterial invasion (Saurabh & Sahoo 2008). One of the noticeable properties of fish lysozyme is the activity against both gram-positive and gram-negative bacteria (Tullio *et al*. 2015). An up-regulation of lysozyme expression was observed in fishes when they exposed to antigenic factors (Hikima *et al*. 2003; Zheng *et al*. 2007; Larsen *et al*. 2009). Yazawa *et al.* (2006) reported that

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the transgenic zebrafish mortality reduced when c-type chicken lysozyme was expressed after 15h (Yazawa *et al*. 2006).

Although, the role of lysozyme in fish immunity has been proved, but unraveling the biochemical properties of lysozyme and its background(s) in innate immunity of fishes seem to be a priority.

Caspian kutum, *Rutilus kutum*, Kamensky 1901, is a fish belonging to the family of Cyprinidae and is widely distributed in the Eurasia, the Black and Azov sea basins, Volga, Atrak, Kuban and Don rivers, the southern and rarely in the northern parts of the Caspian Sea. Little studies have been performed on the immune factors of this fish. In the present study, the lysozyme of Caspian kutum was purified to characterize its biochemical properties and structural changes in the presence of various concentrations of the metal ions. In addition, structural changes of the enzyme in the presence of various concentrations of metal ions and denaturing agents were determined by florescence spectroscopy.

MATERIALS AND METHODS

Animal materials

Caspian kutum was purchased alive from a seafood market in Anzali and Rasht cities, north of Iran. The head kidney was takenand frozen immediately in liquid nitrogen, then stored at −70.0°C for further examinations.

Chemicals

Ethylenediaminetetraacetic acid (EDTA), bovine serum albumin (BSA), glycine and *Micrococcus lysodeikticus* were purchased from Sigma-Aldrich Co. Coomassie Brilliant Blue R-250 and UNOsphere™S cationic exchange resin were obtained from Bio-Rad Laboratories (Hercules, CA, USA). Other materials used in this study was analytical grade employing without any change.

Enzyme extraction and purification

To prepare crude extract, head kidney of the fish was mixed with 100mM phosphate buffer (pH 7.5) with ratio of 1g per 5mL and homogenized for 4min in a homogenizer. The mixture was stirred for 3-4h at 4.0°C and then centrifuged at 8150×g for 15min at 4.0°C. The supernatant (crude extract) was concentrated through a 3kDa cut-off cellulose acetate membrane (Millipore, Molsheim, France) and stored at −20.0°C.

The prepared solution became saturated to 85% (w/v) with a gradual addition of solid ammonium sulphate to the crude extract with gentle stirring at 4.0°C and then 85% saturation ammonium sulfate solution was centrifuged at 8150×g for 15min at 4.0°C. The residue sediment of 85% saturation was dissolved in the minimum volume of 50mMTris-HCl (pH 8.5) and dialyzed against one litre of the mentioned buffer three times at 4.0°C for 6h. The dialyzed solution was centrifuged as mentioned above and then, the obtained supernatant was concentrated through a 3kDa cut-off cellulose acetate membrane.

The resultant extract was loaded on UNOsphere™S column (1.5×20cm) which had been previously equilibrated with 50mMTris-HCl (pH 8.5) (buffer A). Unbound proteins were then removed by washing the chromatography column with the buffer A. To purify bound proteins, the column was washed by sodium chloride (0.2–1M, buffer B). All obtained fractions were assayed for the presence of lysozyme. These fractions were concentrated through a 3kDa cut-off cellulose acetate membrane and stored at -20.0°C. Eventually, the evaluation of protein concentration was carried out using Bradford assay.

Polyacrylamide gel electrophoresis

Protein samples were assayedby SDS-PAGE using a 7% (w/v) stacking gel and 15% (w/v) resolving gel. Subsequently, protein bands were stained with Coomassie Brilliant Blue R-250. Molecular weight of the purified enzyme was measured using SDS-PAGE.

Lysozyme activity assay

Lysozyme activity was assayed according to Shugar's method (Shugar 1952) using *M. lysodeikticus* cell suspended in phosphate buffer 0.1M at pH 6.0. The activity was measured by linear decrease in absorbance at 450nm at 25.0°C over the period of 5min. One unit of activity was defined as the amount of the decrease in optical density of 0.001/min of *M. lysodeikticus*.

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Enzyme kinetics

To determine the kinetic parameters, 0.1mL of enzyme (0.1mg mL^{-1}) was added to 2.5mL volume of the cell suspension of *M. lysodeikticus* at different concentrations (0.002–0.02% w/v). The assays were performed at optimal pH of the enzyme. The Michaelis constant (K_m) and maximum velocity (V_{max}) of the reaction were determined by plotting double reciprocal plot according to the Lineweaver–Burk equation.

Effect of pH on lysozyme activity and stability

Optimal pH for lysozyme activity was measured using pH range of 3 to 8.Mixed buffer (0.2M Na_2HPO_4 acidified with 0.1M citric acid) was employed to control pH. The highest activity was considered as 100%. The rate (%) of relative activity was calculated as a fraction of this value. To determine stability, the enzyme activity was assessed in the acquired optimal pH after incubation in100mM sodium acetate buffer (pH 3.0-5.0), 100mM Na_2HPO_4/citric acid (pH 5.5-7.5), and 100mMTris/HCl (pH 8.0–10.0) for 30 min.

Temperature profile and stability

To determine optimal temperature, the enzyme activity was measured at different temperatures (25.0-70.0°C) at the optimum pH. The temperature in which the enzyme exhibited the maximum activity was considered as 100%, and the relative activity rate (%) were calculated as a fraction of this value.

Then, the relative activity rate (%) was plotted against different temperatures. To determine thermos-stability, the enzyme was incubated at different temperatures ranging from 25.0 to 95.0°C for 30min at the optimum pH. After cooling on ice, the residual enzyme activity was determined.

Effects of metal ions and denaturing agents on lysozyme activity

The effects of metal ions on the lysozyme activity were determined in the presence of NaCl, KCl, $FeCl_2$, $MgCl_2$, $CuCl_2$, and $CrCl_2$ at various concentrations of 1, 5, 10, 50 and 100mM. Furthermore, the effects of urea and sodium dodecylsulfate (SDS) were studied on the enzyme activity at the aforementioned concentrations. The purified lysozymeactivity in the absence of any additive (in 100mM phosphate buffer, pH 6.0) was considered as 100%.

Fluorescence studies of lysozyme by metal ions and denaturing agents

Fluorescence studies of lysozyme in the presence of NaCl, KCl, $FeCl_2$, $CuCl_2$, $CrCl_2$, urea and SDS were investigated at five concentrations (1, 5, 10, 50 and 100mM) in100mM phosphate buffer at pH 6.0. The fluorescence emission spectra were recorded in the range of 200-800nm upon excitation at 280nm. Scanning rate was 500nm/min. The fluorescence emission of the purified lysozyme in the absence of any additive (in 100mM phosphate buffer, pH 6.0) was considered as 100%.

RESULTS AND DISCUSSION

Enzyme extraction and purification

In this study, lysozyme was extracted from the head kidney, as a leukocyte-rich key organ in immune system (Saurabh & Sahoo 2008) of Caspian kutum. To prevent from protease activities, the extraction was performed with cooled buffers. Total activity and protein content of crude extract were 280 units and 371.48mg at 45.0°C and pH 6.0, respectively. Rapid separation of lysozyme was performed by two common steps: ammonium sulphate precipitation and cationic exchange chromatography.

After each step, filtration was carried out by 3kDa cut-off membrane. The results of enzyme purification are summarized in Table 1. After ammonium sulphate (85% saturated) precipitation, the proteins content and enzyme activity were 160.5mg and 200 $Unitsmin^{-1}$, respectively.

The protein solution was loaded onto UNOsphere^{TM}S column. Elution process was carried out with sodium chloride in five steps at 0.2, 0.4, 0.6, 0.8 and 1M NaCl. High activity of lysozyme was observed in fractions 2 and 3 through the cationic column at 0.4M NaCl (Fig.1 a).

The specific activity was determined from 0.75$unitsmg^{-1}$ in the crude extract to 49.29$unitsmg^{-1}$ in the final step. The activity of the enzyme (49.29 units mg^{-1}) was more than *Brosmebrosme* (Ascanius 1772)(40units mg^{-1}) and less than purified lysozymes from *Molva molva* (Linnaeus 1758) (85units mg^{-1}) (Lie *et al.* 1989).

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Table 1. Purification steps of lysozyme enzyme extracted from *Rutilus kutum*, Kamensky 1901.

Purification steps	Total protein (mg)	Protein recovery (%)	Total activity (Units)	Activity recovery (%)	Specific activity (U mg^{-1})
Crud enzyme	371.48	100	280	100	0.75
85 % $(NH_4)_2SO_4$ precipitation	160.5	43	200	71.42	1.24
Cation exchange chromatography	1.42	0.38	70	25	49.29

Polyacrylamide gel electrophoresis

To determine the purity and approximate molecular mass of the purified lysozyme, the SDS-PAGE method was performed. The low-range protein molecular mass markers with 7 bands ranging from 14.4–116kDa from Bio-Rad, were used as standards to calculate molecular mass. Approximate molecular weight of the purified enzyme was estimated as 15.8kDa by SDS-PAGE (Fig.1b). Our results were consistent with the previous reports on the molecular masses of c-type lysozyme from *Asian sea bass* (16.02kDa) (Fu *et al*. 2013), turbot (*Scophthalmus maximus*; 15.7kDa) (Yu *et al*. 2013) and blue shrimp (15kDa) (Mai & Hu 2009). Molecular masses of lysozymes from various animal sources are different (from 11 to 21kDa) (Lockey&Ourth 1996; Nilsen *et al*. 1999; Xue *et al*. 2004).

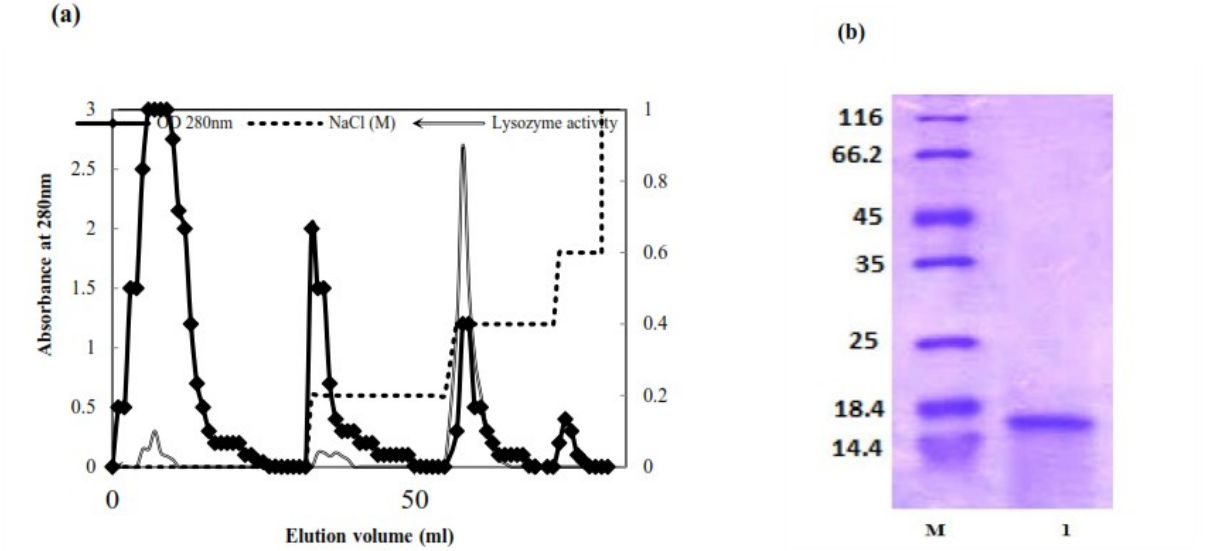

Fig. 1. (a) Cation exchange chromatography of 85% $(NH_4)_2SO_4$ saturation fraction and **(b)** Polyacrylamide electrophoresis is the analysis of the purified lysozyme from *Rutilus kutum*. Column M illustrates marker, and column1 exhibitspurified lysozyme enzyme**.**

Enzyme characterizations

Effect of pHand temperature on the enzyme activity and stability

The pH profile of the purified lysozyme was determined in the pH range of 3.0-8.0 (Fig.2a). The most activity was observed in the pH range of 5.0-7.5 with the maximum activity at pH 6.0. Our results were consistent with the previous finding on grass carp, *Ctenopharyngodon idellus* with the optimal pH range of 5.5 to 7.5 and the optimal pH 6.5 (Ye *et al*. 2010). In some fish and shellfish species, pH profiles have been reported as follows: turbot, *Scophthalmus maximus*, with the pH range of 4.0 to 10.0 and the optimal pH 6.0 (Yu *et al*. 2013), Japanese flounder with the pH range of 4.5 to 6.5 and optimal pH 6.0 (Hikima *et al*. 2001), rainbow trout with the pH range of 4.5 to 6.5 and optimum pH 5.5 (Grinde *et al*. 1988) and Kuruma shrimp with the pH range of 6.0-9.0 (Hikima *et al*. 2001). Klomklao *et al.* reported that the changes in pH may lead to change in the charge distribution and conformation of both substrate and enzyme molecules. These changes disturb the correct positioning of the substrate on enzyme active site. Furthermore, in strong acidic or alkaline pH solutions, most enzymes are denatured irreversibly, thereby decreasing enzyme activity (Klomklao *et al*. 1988; Purich *et al*. 2010).In the present study, the stability of the purified lysozyme in relation to pH is illustrated in Fig. 2b. It was stable over a wide pH range of 3.0 to 10.0which is similar to the purified lysozyme from *Filipino venus* (pH 2.0-11.0) (Kim *et*

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al. 2012) and eastern oyster lysozyme (pH 2.0-13.0) (Xue *et al*. 2004). The pH stability of the purified lysozyme was higher in comparison with pH stability at scallop lysozyme (pH 5.0-6.0) (Kim *et al*. 2012) and the Arctic scallop (pH 5.5-6.6).

In the present study, the influence of temperature on the activity of acquired lysozyme was examined in the range of 25.0-70.0°C using *M. lysodeikticus* as a substrate at pH 6.0 (Fig. 2c). The optimal temperature for the enzyme activity was 45.0°C. The enzyme exhibited60% and 58 % of the maximum activity at 30.0°C and 60.0°C, respectively. Our result was similar to the purified enzyme from rainbow trout, *Oncorhynchus mykiss* with optimal temperature of 45.0°C (Grinde *et al*. 1988). The optimal temperatures for some species have been reported at 25.0°C for Japanese flounder (Hikima *et al*. 2001), 30.0°C for European sea bass, *Dicentrarchus labrax*, (Buonocore *et al*. 2014), 37.0°C for turbot, *S.maximus* (Yu *et al*. 2013) and 60.0°C for grass carp, *C.idellus* (Ye *et al*. 2010).

In the present study, thermo-stability of the purified lysozyme was also examinedby incubation of the enzyme at the various temperatures (25.0-95.0°Cfor 30min). As illustratedin Fig. 2d, at the temperatures higher than45.0°C the purified lysozyme maintained just lower than 50% of its activity, while the enzyme activity reduced strikingly to 29% at 65.0°C and the negligible activity was observed at 85.0°C (only 5%). Moreover, the purified lysozyme was completely inactivated at 95.0°C after 30 min. Purified lysozyme from *Filipino venus* (Kim *et al*. 2012)retained 55% of its activity after being in 80.0°C. Our results exhibitedthat the purified lysozyme from Caspian kutum is not heat-stable.

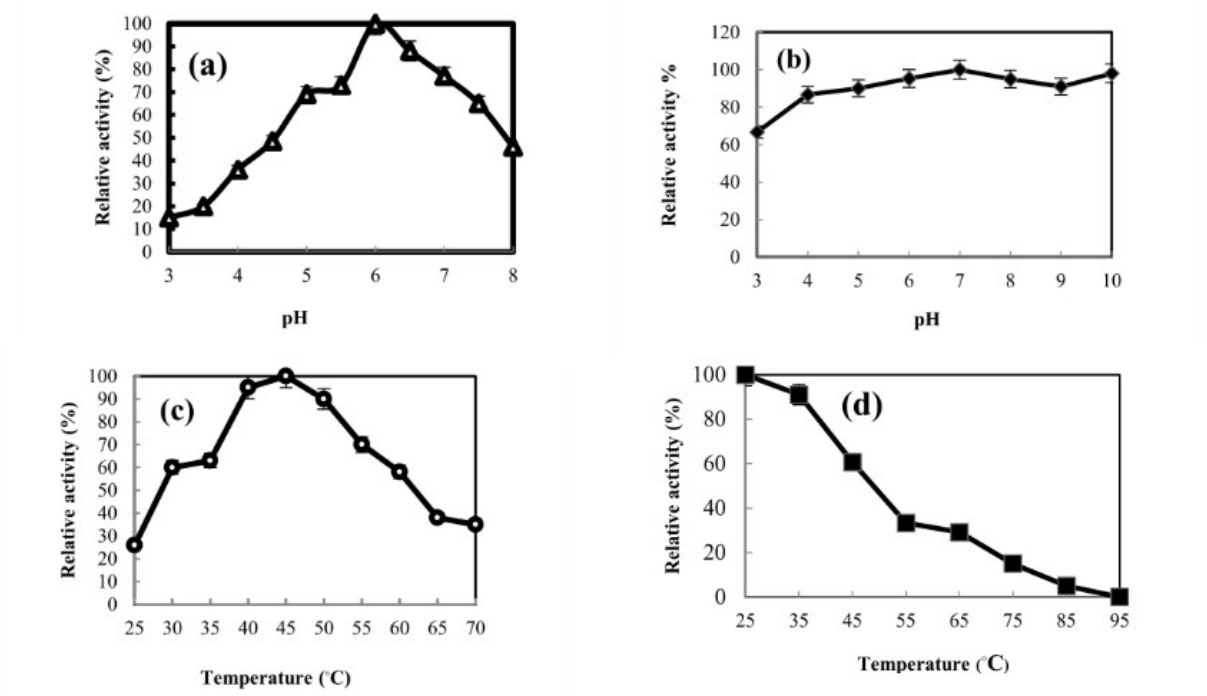

Fig. 2. Effects of pH and temperature on activity and stability of the purified lysozyme of *Rutilus kutum*. (a) The highest activity of lysozyme was observed at pH 6.0. (the optimum pH), (b).The determination of stability was performed in presence of 100mM sodium acetate buffer (pH 3–5), 100mM Na_2HPO_4/citric acid (pH 5.5–7.5) and 100mMTris/HCl (pH 8–10). **(c)** The highest (100%) activity of lysozyme was observed at 45.0°C (d) Thermo-stability of lysozyme was determined by incubating enzyme at various temperatures ranging from 25.0 to 95.0°C for 30 min in 100mM phosphate buffer at pH 6.0.

Enzyme kinetics

The kinetic constants (V_{max} and K_m) of our purified lysozyme from *R.kutum* were calculated by a graphical representation of the Lineweaver–Burk plot. The K_m and V_{max} values of the enzyme towards *M. lysodeikticus* as substrate were 0.007g/100mL (corresponding to 0.00007g mL^{-1}) and 200units/min, respectively (Fig. 3).

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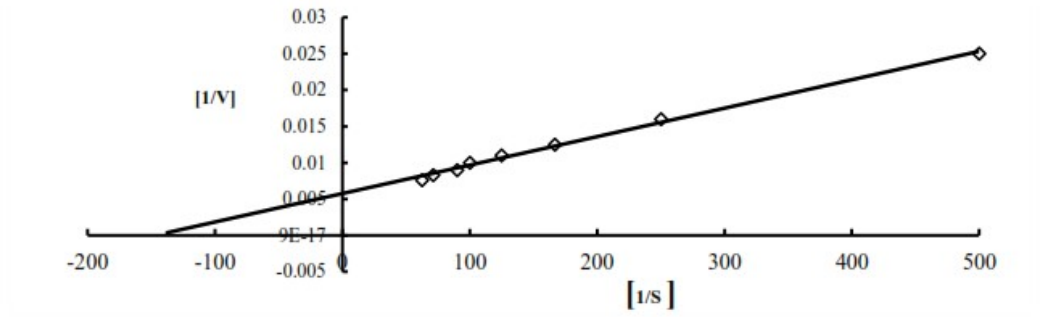

Fig. 3. Kinetic analysis of the purified lysozyme from *Rutilus kutum*. K_m and V_{max} were determined by plotting Lineweaver-Burk plot of the enzyme activity (units min^{-1}) against various concentrations of *M. lysodeikticus* (g/100mL).

Effects of metal ions and denaturing agents on lysozyme activity

The effects of different mono- and divalent cations on the activity of lysozyme from Caspian kutum is presented in Fig.4a. Enzyme activity continuously elevated by raising the NaCl concentrations from 0 to 50mM, while at the higher NaCl concentrations, the levels of activity decreased. The lysozyme activity raised in other animal species such as eastern oyster at 0 to 100 mM NaCl (Xue *et al*. 2004), *Filipino venus* at 0 to 70mMNaCl (Kim *et al.* 2012) and egg-white lysozyme from hen eggs at 0 to 90mMNaCl(Walter *et al*. 1972)and then reduced with further elevating in NaCl concentrations. Lysozyme activity increased in the range of 1–10mMKCl to approximately 150% of its initial activity. At the higher KCl concentrations, the activity decreased to 75% of its initial activity. Pervious finding in other animal species exhibited that lysozyme activity from *Filipino venus* was constant in the range of 10–50mMKCl, while decreased significantly by increase in concentrations to higher than 50mM (Kim *et al*. 2012). As illustrated in Fig. 4a, lysozyme activity was significantly decreased in the presence of $FeCl_2$ and $CuCl_2$. It was reduced to 35% and 0% after incubation in 10mM$FeCl_2$ and higher concentrations, respectively. Our results displayedthat the enzyme was completely inactivated at 50mM $FeCl_2$. Its activity decreased sharply even in the presence of 1mM $CuCl_2$containing buffer, while in 10-100mM $CrCl_2$reduced and even exhibited only 5% at 100mM.The enzyme was not stable in the presence of $MgCl_2$ (1-100mM) and the activity continuously changed.So that, the high activity was observed in the presence of 50mM, then was completely inactivated at 100mM.

As illustrated in Fig. 4b, the lysozyme activity reduced in the presence of SDS. The purified lysozyme from Caspian kutum displayed lower than 50% of its activity at the concentrations higher than 1mM SDS and completely inactivated at 50mM. In 1-100mM urea, unlike SDS, its activity retained higher than 50%.

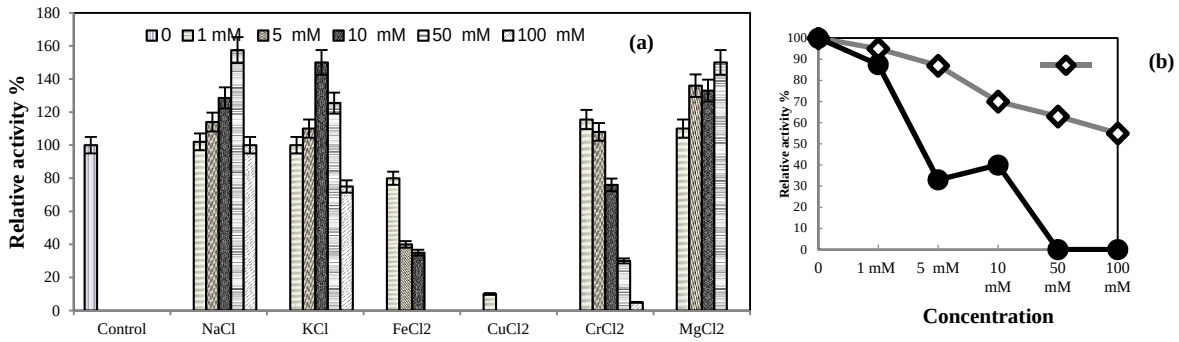

Fig. 4. Effects of metal ions and denaturing agents on activity of the purified lysozyme of *Rutilus kutum*. (a) Effects of NaCl, KCl, $FeCl_2$, $MgCl_2$, $CuCl_2$, and $CrCl_2$, and also (b) SDS and urea at 1, 5, 10, 50 and 100mM in100mM phosphate buffer at pH 6.0 on the activity of purified lysozyme.

Intrinsic fluorescence

As illustrated in Fig.5a, by measuring lysozyme emission intensity, we tracked changes in the spectra of lysozyme by plotting the fluorescence intensity at 340nm against 1, 5, 10, 50, 100mM of NaCl, KCl, $FeCl_2$, $CuCl_2$ and $CrCl_2$.

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In general, the emission intensity increased in the presence of NaCl, KCl, $CrCl_2$ (Fig.5a**)** and SDS, while it was negligible in the urea (Fig. 5b).

The fluorescence quenching of purified lysozyme was observed in the presence of $FeCl_2$ and $CuCl_2$(Fig.5a). $FeCl_2$ effect on the fluorescence quenching of lysozyme had more intensity than $CuCl_2$, so that in the presence of 1mM $FeCl_2$ only 11.07% of emission remained. In addition to decrease in fluorescence of the lysozyme in the presence of $FeCl_2$ and $CuCl_2$ at 340 nm, new spectra with various maxima were produced. So that, the lysozyme-$CuCl_2$ spectrum at concentrations ≥ 5mM and lysozyme-$FeCl_2$ spectrum at concentrations ≥1M had a maximum at 425nm and 440nm, respectively. Fig.5c exhibits the fluorescence spectrum of the $CuCl_2$ in the range of 300-460nm. The peak at 425nm corresponding to the right sift is attributed to 5mM $CuCl_2$.

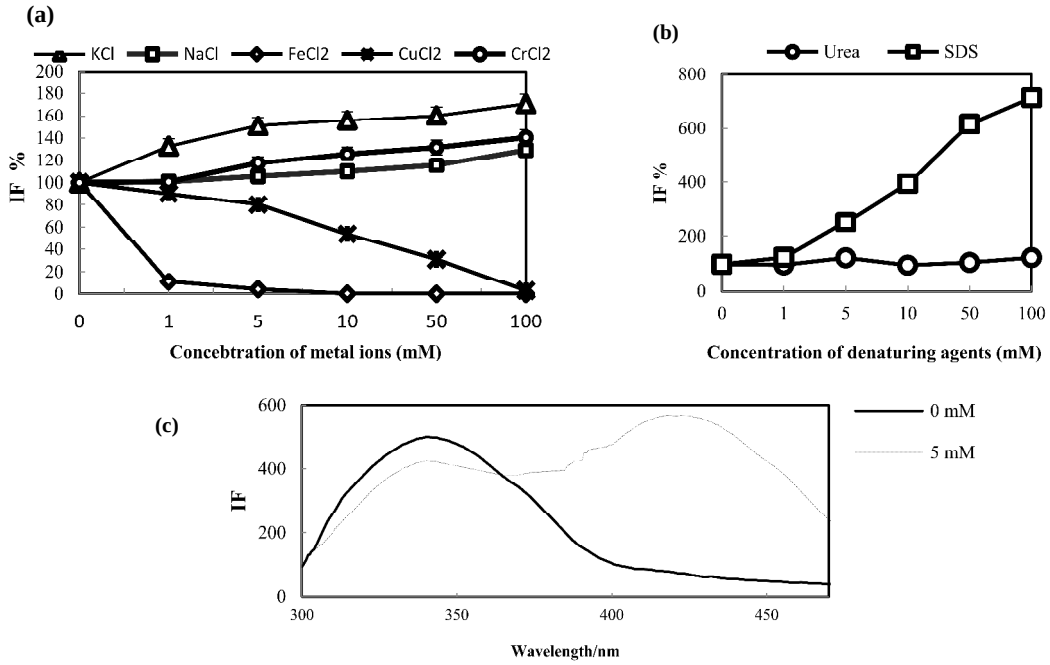

Fig. 5. Effects of metal ions and denaturing agents on the fluorescence emission lysozyme of *Rutilus kutum*. (a)The effects of NaCl, KCl, $FeCl_2$, $CuCl_2$, and $CrCl_2$, as well as (b) SDS and urea at the various concentrations of 1, 5, 10, 50 and 100mM on the fluorescence emission of lysozyme. (c)The fluorescence spectrum of the $CuCl_2$ in the range of 300-460nm. The peak at 425nm corresponded to the right sift is attributed to 5mM $CuCl_2$.

Our results exhibit that the fluorescence intensity of lysozyme is proportional to the different concentrations of metal ions and denaturing agents except for urea. Therefore, the overall purified lysozyme fluorescence spectrum can change by interaction with metal ions and SDS, indicating the sudden condition changes of the tryptophan residues. In other words, hydrophobic tryptophan residues can relocate from the interior of the protein upon exposing to the polar solvent. As aforementioned, we observed new wavelength maxima of 425nm and 440nm, oncelysozyme was exposed to $FeCl_2$ and $CuCl_2$ respectively, hence these wavelength areas shift the emission spectrum to the right. Di Stasio (2004) reported that the increases in solvent polarity, intensify energy relaxation, and can result a shift in the emission spectrum to the right. In the present study, it is also observed that a pronounced fluorescence quenching can occur in the purified lysozyme upon addition of $FeCl_2$ and $CuCl_2$, indicating that $FeCl_2$ and $CuCl_2$ quenched the exposed tryptophan of the purified lysozyme. Our result exhibited that the purified enzyme displays a little conformation change in the presence of 1-100mM urea. Since at 100mMurea, lysozyme retained higher than 50% of its activity, expectedly, pure lysozyme protein has a degree of resistance to urea. In fact, lysozyme structure does not enter into strong interactions with urea, becausethe interactions between urea and protein are considered as a main factor in the mechanism of protein denaturation by urea.

The interactions between metal ions and denaturing agents could induce structural alterations on the active site of the lysozyme or on its proximity, hence, be effective on the antibacterial activity of this enzyme. So that, our result exhibited that NaCl and KCl are well-established metal ionsin elevating the purified enzyme activity. However,

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the activity was reduced significantly in the presence of $FeCl_2$, $CuCl_2$, $CrCl_2$ and SDS. Therefore, the structural alterations induced by $FeCl_2$, $CuCl_2$, $CrCl_2$ and SDS can exert negative effects on the anti-bacterial activity of purified lysozyme extracted from Caspian kutum. The high $MgCl_2$concentrations also exert the negative effects on the enzyme activity. Thus, it can be suggested that the purified enzyme structure can be made more stable by medium engineering through make change the salt composition of its aqueous solution.

CONCLUSION

This study characterized some of the biochemical qualities of lysozyme enzyme extracted from Caspian kutum (*Rutilus kutum*, Kamensky 1901).The enzyme was purified by ion-exchange chromatography with specific activity 0.75 unitmg^{-1} in the crude extract to 49.29units mg^{-1} in the final step with activity recovery of 25%. Approximate molecular weight of the purified Lysozyme was estimated as 15.8kDa by SDS-PAGE. The enzyme exhibited the optimum activity at the pH range of 5.5 to 7.5 with the optimum pH of 6.0, and was stable around a wide pH range of 3.0 to 10.0. The optimum temperature for the enzyme activity was determined at 45.0°C with the range of optimum temperature 30.0 to 60.0°C. Thermostability of enzyme was low at the higher temperatures than 50°C.The purified lysozyme showed low stability against surfactants such SDS. The enzyme activity was significantly decreased in the presence of $FeCl_2$, $CuCl_2$ and $CrCl_2$. Moreover, the purified lysozyme was completely inactivated at the concentration of 100mM $MgCl_2$. It can be suggested that the enzyme is sensitive to the changes of the metal ions concentrations, and also is not heat stable. Therefore, some phenomena such as water hardness and water temperature fluctuations in the living environment of Caspian kutum can have negative effects on its innate immunity.

In the years between 1950 and 1970, Caspian kutum catches is recorded over 30,000 tons per year in Anzali Wetland. The total catch of Caspian kutum was decreased almost tenfold during the last 50 years (http://www.hamshahrionline.ir/details/74792). This significant decrease is largely attributed to the anthropogenic impacts on the southern coasts of the Caspian Sea, which led to a lot of changes in the living habitat of Caspian kutum, including the destruction and fragmentation of its habitats and the change in the properties of its environments. Basic studies similar to this study can play an important role in understanding the biology and behavior of this fish in the face of conditions such as changes in the physical properties of water; change in water temperature, hardness, and pH.

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خصوصیات بیوشیمیایی لیزوزیم استخراج شده از دریای خزر، *Rutilus kutum*, Kamensky 1901

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چکیده

این مقاله، یافته‌های یک مطالعه‌ی پایه روی ویژگی‌های بیوشیمیایی و فعالیت آنزیم لیزوزیم استخراج شده از یک ماهی تجاری مهم به نام ماهی سفید دریای خزر (*Rutilus frisii kutum*، Kamensky، 1901) را ارائه می‌دهد. آنزیم با روش رسوب‌دهی آمونیم سولفات و کروماتوگرافی تبادل کاتیونی استخراج شد. وزن مولکولی لیزوزیم توسط SDS-PAGE ۱۵,۸کیلودالتون محاسبه شد. pH و دما بهینه به ترتیب ۶,۰ و ۴۵,۰ درجه سانتیگراد بود و مقادیر K_m و V_{max} به ترتیب (۰,۰۰۰۰۷ ($g\ ml^{-1}$ و (units/min) ۲۰۰ بود. پایداری حرارتی آنزیم در دماهای بالاتر از ۵۰ درجه سانتی‌گراد پایین بود. فعالیت آنزیمی در حضور NaCl (۱-۵۰ میلی مولار)، KCl (۱-۵۰ میلی مولار)، و $CrCl_2$ (۱-۵ میلی مولار)، افزایش یافت، سپس در غلظتهای بالاتر کاهش یافت. علاوه بر این، فعالیت آنزیم در حضور $FeCl_2$ (۱-۱۰۰ میلی مولار)، و CuCl2 (۱-۱۰۰ میلی مولار)، به طور قابل توجهی کاهش یافت و فعالیت آنزیم در حضور $MgCl_2$ پایدار نبود. فعالیت آنزیم توسط SDS مهار شد، اما آنزیم مقاومت خوبی نسبت به اوره داشت. کوانچینگ فلورسانس در حضور $FeCl_2$ و $CuCl_2$ مشاهده شد. شدت نشر فلورسانس در حضور NaCl، KCl، $CrCl_2$ و SDS افزایش یافت، در حالی که شدت نشر فلورسانس برای اوره ناچیز بود. نتایج ما نشان می‌دهد که NaCl و KCl یون‌های فلزی خوب- تثبیت شده هستند که می توانند فعالیت آنزیم را افزایش دهند، اما لیزوزیم تخلیص شده پایداری متوسطی را نسبت به یون‌های فلزی دیگر نشان نداد، همچنین لیزوزیم تخلیص شده از ماهی سفید دریای خزر در برابر حرارت پایدار نبود. بنابراین برخی از پدیده‌هایی مانند سختی آب و گرم شدن می‌توانند تاثیرات منفی بر ایمنی ذاتی این ماهی داشته باشند. می‌توان پیشنهاد کرد که ساختار آنزیم با استفاده از روش مهندسی محیط از طریق تغییر دادن ترکیبات نمک محلول آبی می‌تواند پایدار شود.

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