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BIOCHEMICAL CHEMISTRY

Effect of Magnesium Cations on the Activity and Stability of β-Galactosidases

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Abstract—It was shown that the presence of magnesium cations in the reaction mixture increases, approximately twofold, the activity of bacterial *Escherichia coli* and yeast *Kluyveromyces lactis* β-galactosidases but does not affect the activity of bovine liver and fungous *Penicillium canescens* β-galactosidases. The catalytic constants for *E. coli* and yeast *K. lactis* β-galactosidases in the presence of 0.01 M and in the absence of Mg^{2+} cations were determined (490 and 220 s^{-1} and 59.8 and 37.4 s^{-1}, respectively). It was shown that the Michaelis constants for these two enzymes are higher in the presence of Mg^{2+} cations, that the thermal stability of *E. coli* and *K. Lactis* β-galactosidases is higher in the presence of 0.01 M Mg^{2+}, and that the effective rate constants of thermal inactivation of the enzymes are two- to eightfold lower, depending on conditions, in the presence of Mg^{2+} cations. The maximum stabilizing effect of magnesium cations was observed at weak alkaline pH values (7.5–8.5).

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β-Galactosidases (EC 3.2.1.23) are enzymes that participate in hydrocarbon metabolism of living organisms; they are isolated from plants, microorganisms, and organs of humans and animals. β-Galactosidases are classified as glycosyl hydrolase families 1, 2, 35, and 42 of the GH-A class that includes β/α-proteins, catalytically active sites of which are residues of glutamic acid [1]. The catalytic activity of enzymes belonging to family 2 is determined by the presence of metal cations: Mg^{2+}, Na^{+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Al^{3+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, and Ag^{+}. Depending on enzyme and reaction conditions, the cations may promote or inhibit the catalytic activity of β-galactosidases [2–4]; the published data on the effect of metal cations on the activity of β-galactosidases are controversial. As a rule, magnesium cations enhance the activity of β-galactosidases [5, 6]; however, for some enzymes, e.g., β-galactosidase isolated from bifidobacteria, magnesium cations at concentrations from 1 to 100 mmol/l inhibit the enzyme activity [7]. The presence of magnesium cations in the reacting mixture enhances the activity of bacterial *Escherichia coli* β-galactosidase [5, 8], but if the concentration of magnesium cations exceeds 1 mmol/l, the enzyme activity is inhibited [9].

An X-ray analysis of *E.coli* β-galactosidase showed that the active site of the enzyme contains two Mg^{2+} cations [10]; in addition, there are other sites of binding of Mg^{2+} ions [11]. Three amino acid residues (Glu-461 [12], His-418 [13], and Glu-416 [14]) are ligands of magnesium ions in the enzyme active site; Glu-461 is one of the catalytic groups. Opinions differ as to the role of magnesium cations in the catalytic effect. First of all, Mg^{2+} ions may participate in the catalysis indirectly, by stabilizing a catalytically active protein conformation [15]. In addition, Mg^{2+} ion may play the role of electrophilic catalyst by means of coordination with oxygen of the glycoside bond [16, 17].

The aim of this work is to examine how magnesium cations affect the activity and stability of β-galactosidases of various origins.

EXPERIMENTAL

We studied bacterial, yeast, fungous, and animal β-galactosidases. The properties of these enzymes are listed in Table 1.

The activity of β-galactosidases was determined from the rate of hydrolysis of 2-nitrophenyl-β-D-galactopyranoside at 25°C and pH 4.5 (*P. canescens*) and 7.5 (the other enzymes). In experiments with fungous *P. canescens* β-galactosidase, 0.1 mol/l phosphate-citrate buffers were used; with other enzymes, 0.1 mol/l phosphate buffers. The catalytic and Michaelis constants were determined in the presence (0.01 mol/l) and absence of magnesium cations.

RESULTS AND DISCUSSION

The addition of magnesium salts in concentrations up to 100 mmol/l produced no effect on the catalytic properties of bovine liver and fungous β-galactosidases. As is seen from Table 1, the presence of magnesium cations does not affect the Michaelis and catalytic constants for these enzymes. For other two β-galactosi-

Table 1. Characteristics of the β-galactosidases studied

Origin	c, wt %	pH_{opt}	K_m, mmol/l		k_{cat}, s^{-1}	
			I	II	I	II
Bovine liver	95	7.0–7.5	2.5	2.5	0.056	0.056
Penicillium canescens fungi	15	4.2–4.5	1.0	1.0	35.8	35.8
Escherichia coli bacteria	80	7.0–7.5	0.09	0.25	220	490
Kluyveromyces lactis yeast	20 mg/ml	7.0	1.4	2.0	37.4	59.8

Note: Yeast enzyme was in the form of glycerol solution, while the rest of the enzymes were lyophilized powders. The kinetic parameters were determined in the (I) absence and (II) presence of 0.01 mol/l Mg^{2+}; c is the protein content.

dases, the presence of magnesium cations caused a slight increase in the Michaelis constant and a 1.5- or 2-fold rise in the enzyme activity. Figure 1 shows the activity of *K. lactis* β-galactosidase as a function of the concentration of magnesium cations in the reaction mixture. The maximum increase in the enzyme activity is observed at concentrations of magnesium cations up to 0.15 mmol/l. As the concentration of magnesium ions increases to 1 mmol/l, the enzyme activity reaches a constant level. A similar dependence is observed for *E. coli* β-galactosidase [5].

An X-ray analysis of *E. coli* β-galactosidase showed that two Mg^{2+} cations participates in the formation of its active sites [10]. By now, another two bacterial [18, 19] and one fungous [20] β-galactosidases have been investigated by the XRA method. Of these, only in *E. coli* β-galactosidase, which belongs to the glycosyl–hydrolase family 2 (whose activity is determined by metal cations), magnesium cations participate in the formation of its active site [10]. For *Sulfolobus solfataricus* (family 1) [18], *Thermus thermophilus* A4 (family 42), and *Penicillium* sp. (family 35) [20] β-galactosidases, studied using X-ray analysis, no magnesium cations were detected in the active sites. The activity of β-galactosidases of glycosyl–hydrolase families 1, 35, and 42, to which these enzymes belong, is not determined by metal cations [1].

Yeast *K. lactis* β-galactosidase has not been investigated by X-ray diffraction analysis a, but its amino acid sequence is well-known. A computer-aided comparison of the amino acid sequences of *K. lactis* and *E. coli* β-galactosidases [21] showed that the functional amino acid residues in the catalytic domain and the ligand surrounding of the magnesium cations remain in these both sequences. It was supposed that the activity of *K. lactis* β-galactosidase depends on the presence of magnesium cations in the reacting mixture; the supposition was supported experimentally [21]. A similar result was obtained by the authors of [6] who performed a computer-aided comparison of amino acid sequences of *E. coli* and *Lactococcus lactis* ssp. *Lactis* 7962 β-galactosidases and investigated the effect of magnesium cations on the enzymes activity.

The effect of magnesium cations on the activity of β-galactosidases containing this cation in its active site may be due to the fact that dissolution of the enzyme results in dissociation of magnesium cations from the active site. Since magnesium cations may contribute to the functioning of the β-galactosidase active site [15, 16], their passing from the enzyme active site to the solution results in a partial reversible inactivation of the enzyme. Addition of magnesium salts to the reaction mixture results in a shift of the dissociation equilibrium and restoration of the enzyme activity. Since bovine liver and fungus *P. canescens* are like to contain no magnesium cations in the active site,

Table 2. Effective rate constants of dissociation (k_{1eff}) and denaturation (k_{2eff}) of β-galactosidases in the presence and absence of magnesium cations at various temperatures (phosphate buffer, pH 7.5)

Enzyme	t, °C	$[Mg^{2+}]$, M	$k_{1eff} \times 10^4$, s^{-1}	$k_{2eff} \times 10^4$, s^{-1}
E. coli, 7.3 mg/l	45	0	4.8	0.2
		0.01	0.6	0.1
E. coli, 36.5 mg/l	45	0	4.8	0.6
		0.01	0.6	0.1
E. coli, 7.3 mg/l	48		9.8	1.0
			3.2	0.2
E. coli, 36.5 mg/l	48	0	9.8	1.6
		0.01	3.2	0.2
K. lactis	25	0	3.0	0.3
		0.01	1.0	0.2
K. lactis	35	0	17.3	2.0
		0.01	3.0	0.7
K. lactis	40	0	24.1	4.4
		0.01	10.2	1.9

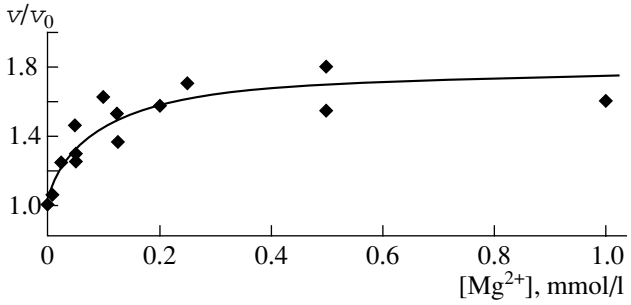

Fig. 1. Dependence of the activity of *K. lactis* β-galactosidase on the content of magnesium cations in the reaction mixture. Phosphate buffer (pH 7.5), 25°C, 2-nitrophenyl-β-D-galactopyranoside substrate; v_0 is the reaction rate in the absence of Mg^{2+}.

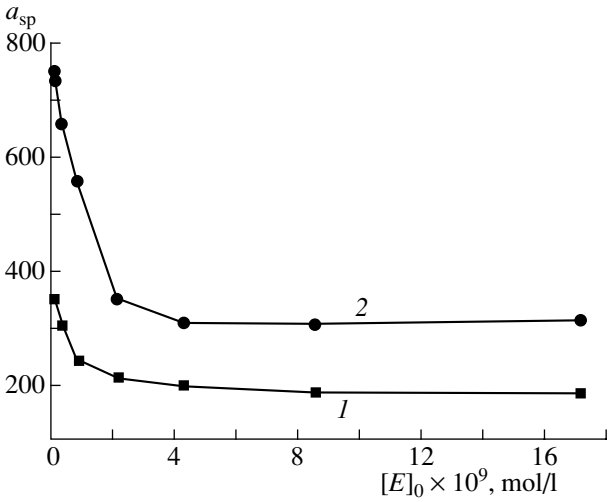

Fig. 2. Dependence of the specific activity (a_{sp}, molecules/active site) of *E. coli* β-galactosidase on the enzyme concentration (*1*) in the absence of magnesium cations in the reaction mixture and (*2*) in the presence of 0.01 mol/l Mg^{2+}.

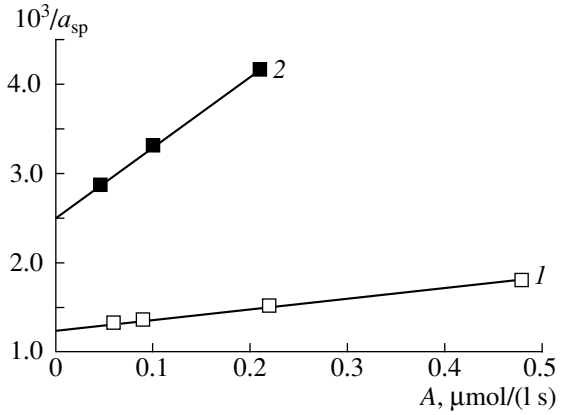

Fig. 3. Determination of the specific activity of dimers of *E. coli* β-galactosidase by linearization of the experimental data obtained (*1*) in the presence of 0.01 M Mg^{2+} and (*2*) in the absence thereof.

addition of magnesium salts to the reaction mixture produces no effect on the activity of the enzyme.

Figure 2 shows the dependence of the specific activity of *E. coli* β-galactosidase on the enzyme concentration in the absence and in the presence of 10 mmol/l Mg^{2+}. Over the entire range of concentrations of the enzyme studied, its activity is higher in the presence of magnesium cations. That the specific activity of the enzyme increases as its concentration decreases is associated with by the formation of more active subunits due to the dissociation of oligomers. For bacterial *E. coli* β-galactosidase, the catalytically active forms are tetramers and dimers; monomers are inactive [10]. The existing dependence of the specific activity of the enzyme on its concentration makes it possible to determine the specific activities of the enzyme oligomers of various compositions [22]. Figure 3 shows the linearized experimental data plotted in the coordinates that make it possible to determine the activity of the enzyme dimers from the intercept at the ordinate. The activity of tetramers and dimers of *E. coli* β-galactosidase in the absence of magnesium cations is 180 and 400 molecules/(s act.site). In the presence of 10 mmol/l Mg^{2+}, the activity increases to 300 and 800 molecules/(s act.site). Thus, the activity of *E. coli* β-galactosidase increases in the presence of magnesium cations because the specific activity of dimers and tetramers of the enzyme increases approximately to the same extent.

The presence of magnesium cations enhances not only the activity of *K. lactis* and *E. coli* β-galactosidases but also their thermal stability. Figures 4 and 5 show the kinetic curves of thermal inactivation of the enzymes plotted in the coordinates of the first-order

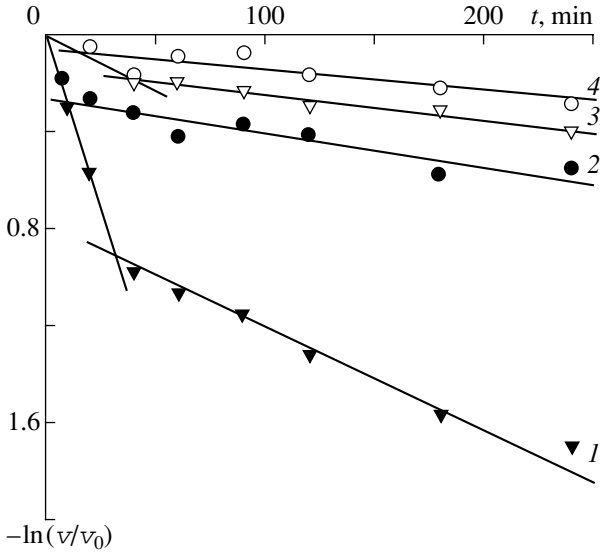

Fig. 4. Kinetic curves of thermal inactivation of *E. coli* β-galactosidase at 45°C (*1*, *2*) in the absence and (*3*, *4*) presence of 0.01 mol/l magnesium cations. Phosphate buffer, pH 7.5, the enzyme concentrations (*2*, *4*) 7.3 and (*1*, *3*) 36.5 mg/l.

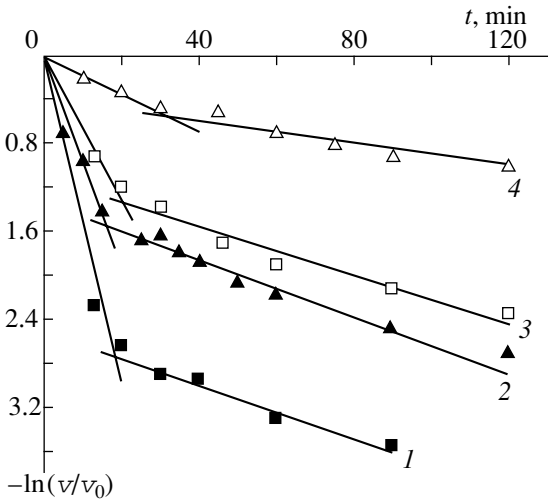

Fig. 5. Kinetic curves of thermal inactivation of *K. lactis* β-galactosidase (*1*, *2*) in the absence and (*3*, *4*) presence of 0.01 mol/l magnesium cations at (*2*, *4*) 35 and (*1*, *3*) 40°C.

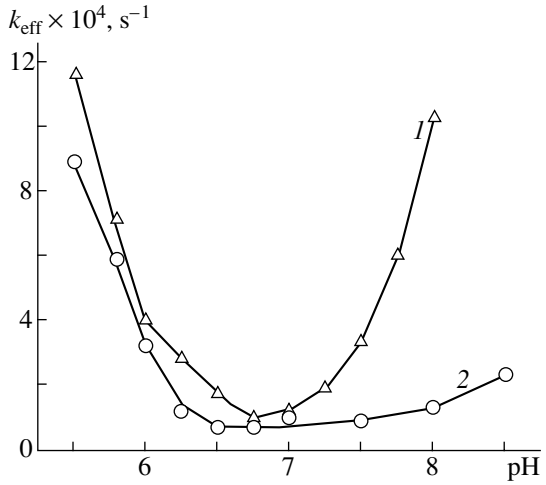

Fig. 6. pH dependences of effective rate constants of inactivation of *E. coli* β-galactosidase (*1*) in the presence and (*2*) absence of magnesium cations. Phosphate buffer, 52°C.

equation for various temperatures and concentrations in the presence and absence of magnesium cations. The inflection in the kinetic curves may point to the dissociative mechanism of thermal inactivation [23]; the effective rate constants of dissociation and denaturation ($k_{1\text{eff}}$ and $k_{2\text{eff}}$) can be determined from the slopes of the two segments of the experimental dependence. The constants values are listed in Table 2.

As can be seen from Table 2 and Figs. 4 and 5, the rate of inactivation of the enzymes decreases in the presence of magnesium cations; the maximum effect of magnesium cations is observed at pH > 7. Figure 6 shows the pH dependence of the effective rate constants of inactivation in the presence and absence of magnesium cations for *E. coli*, β-galactosidase at 52°C. The data show that the rate of inactivation depends drastically on the pH value; an increase in the pH to 8.0 results in a twofold increase in k_{eff} in the presence of magnesium ions and in a tenfold increase in the absence. In addition, the presence of magnesium cations extends the pH range of stability of the enzyme.

The study performed showed that addition of magnesium cations to buffer solutions or reaction mixtures may either enhance the catalytic activity and stability of β-galactosidases (*E. coli* and *K. lactis*) or produce no effect on the properties of the enzyme (bovine liver and fungous *P. canescens* β-galactosidases).

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