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INFLUENCE OF METAL IONS ON THE ACTIVITY AND STABILITY OF THE GLUCOSE ISOMERASE FROM Streptomyces atratus

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The influence of a number of bivalent metals on the activity of Streptomyces atratus glucose isomerase has been studied. Mg^{2+} ions are activators and Co^{2+} ions are stabilizers of the glucose isomerase activity. The effective kinetic parameters for the action of the enzyme have been determined.

It is known that the activity of glucose isomerases depends on the presence in the reaction mixture of several metal cations that are cofactors of the enzyme. In the main, the maximum activity of glucose isomerase is shown in the presence of Co^{2+} and Mg^{2+} ions [1]. However, so far as concerns the glucose isomerase of Lactobacillus brevis 74, the greatest activating action is exerted by the simultaneous presence of cobalt and chromium ions [2]. For the majority of glucose isomerases from various sources the main activators are Mg^{2+} ions, while Co^{2+} ions play the role of stabilizers [3-6]. It is known from the same sources that the maximum activating effect is observed under the simultaneous action of Mg^{2+} and Co^{2+} ions. Values of K_M for Mg^{2+} and Co^{2+} ions have been calculated for glucose isomerases from various microorganisms [1].

The aim of the present paper is to describe results relating to the influence of metal ions on the enzymatic activity of Str. atratus glucose isomerase.

The presence of Mg^{2+} ions and, to a smaller degree, Co^{2+} ions is necessary for the performance of the isomerism of aldosugars into ketosugars with the participation of the Str. atratus glucose isomerase. Elimination of metal ions from the enzyme solution by dialysis against a buffer containing 0.01 M EDTA led to the complete disappearance of enzymatic activity.

On the performance of similar studies with the glucose isomerase of L. brevis 74, Toluelova et al. [2] established that the enzyme was weakly bound by a metal and, consequently, no covalent bonds exist between protein and metal.

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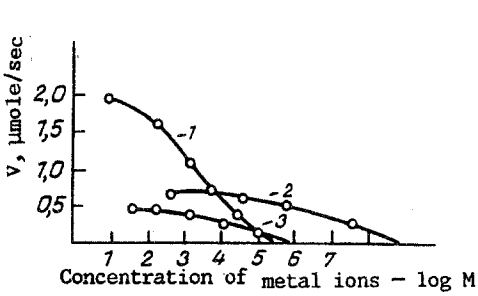

Fig. 1

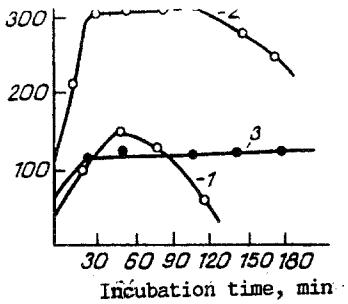

Fig. 2

Fig. 1. Influence of various bivalent metal ions on the activity of glucose isomerase: 1) Mg^{2+}; 2) Co^{2+}; 3) Mn^{2+}.

Fig. 2. Influence of Mg^{2+} and Co^{2+} on the activity of the Str. atratus glucose isomerase previously incubated with a 0.01 M solution of EDTA (1), and in the native state (2, 3): 1) $[Mg^{2+}]$ = 0.1 M + $[Co^{2+}]$ = 1 mM; 2) $[Mg^{2+}]$ = 0.1 M; 3) $[Co^{2+}]$ = 1 mM; [E] = 0.05 mM, [S] = 0.1 M, pH 7.8.

TABLE 1. Influence of Some Metal Ions on the Activity of the Str. atratus Glucose Isomerase

Metal salts	Residual activity, %	Metal salts	Residual activity, %
Without Me^{2+}, + ions	0	$MnCl_2$	50 of the activity with $MgSO_4$
$MgSO_4$	100	$CoCl_2$	70 "
$ZnSO_4$	40 of the activity	$HgCl_2$	51 "
$FeSO_4$	35 with $MgSO_4$	$PbCl_2$	41 "
$BaSO_4$	47 "	$NiSO_4$	45 "
$CuSO_4$	15 "	$AgNO_3$	7 "
$CaCl_2$	28 "		

We have studied the influence of various bivalent metal ions on EDTA-treated Str. atratus glucose isomerase. The strongest activation of the isomerization of D-glucose into D-fructose was observed in the case of Mg^{2+} ions, a less strong one with Co^{2+} ions, and a very weak one with Mn^{2+} ions (Table 1). Almost no effect on enzymatic activity was exerted by Fe^{2+}, Ni^{2+}, Ca^{2+}, Zn^{2+}, and Cd^{2+} ions, while Hg^{2+} and Ag^{+} ions showed an inhibiting action.

From the results presented in Fig. 1 it can be seen that the activating action of Mg^{2+} ions on Str. atratus glucose isomerase is stronger than that of Co^{2+} ions. The activating effect of the ions depends on their concentration. It has been established that the optimum concentration for the activation of Str. atratus glucose isomerase by Mg^{2+} ions is 0.1 M, and by Co^{2+} ions 1 mM. At higher concentrations these ions exerted an inhibiting action.

Furthermore, the action of the metal ions also depends on the temperature. The activating activity of cations begins to be displayed at 40°C and reaches a maximum at 60-70°C. Figure 2 shows results on the change in glucose isomerase activity of the native enzyme and the enzyme that had been treated with EDTA solution both of which had first been incubated at 75°C for 1-3 h with the ions Mg^{2+} (0.1 M) and Co^{2+} (1 mM) together and separately. It can be seen that Mg^{2+} and Co^{2+} ions restored the activity lost when the enzyme was treated with EDTA solution. Without the combined addition of Mg^{2+} and Co^{2+} ions the EDTA-treated enzyme exhibited no activity [sic], while the additions of a mixture of Mg^{2+} and Co^{2+} ions in concentrations of 0.1 M and 1 mM led not only to the activation but also to the stabilization of the enzyme (Fig. 2, curve 1).

Other results were obtained in experiments with the native enzyme (without EDTA treatment). Preliminary incubation of the native enzyme with solutions of salts of Mg^{2+} (0.1 M) or Co^{2+} (1 mM) (Fig. 2) showed that the activating action of Mg^{2+} lasted for 2 h, after which there was a rapid inactivation of the enzyme, while when only Co^{2+} ions or Mg^{2+} and Co^{2+} were present in a reaction mixture at 75°C the activity of the enzyme was retained for 24 h. On the basis of the results obtained it may be concluded that Str. atratus glucose isomerase, unlike glucose isomerases from other sources [7], is a Mg^{2+}-dependent enzyme,

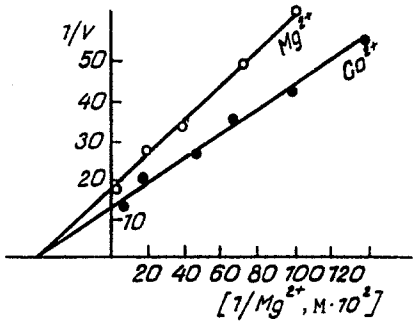

Fig. 3

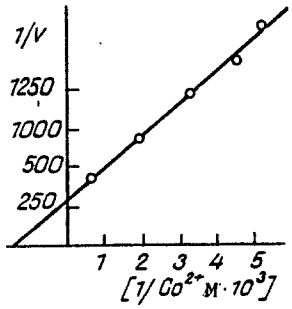

Fig. 4

Fig. 3. Graph of double reciprocal values of the dependence of the rate of isomerization of D-glucose by the enzyme at various concentrations of Mg^{2+} ions in the absence and in the presence of Co^{2+} ions.

Fig. 4. Determination of the parameters of the activation by cobaltous chloride of the isomerization of D-glucose by the native glucose isomerase in the Lineweaver–Burk coordinates: [S] = 0.1 M; [E] = 0.06 mg/ml in 0.05 M phosphate buffer, pH = 7.8.

TABLE 2. Kinetic Characteristics of the Isomerization of D-Glucose into D-Fructose under the Influence of Different Concentrations of Mg^{2+} and Co^{2+} Ions

$[Mg^{2+}]$	$[Co^{2+}]$	K_M	K_{cat} min^{-1}	K_{cat}/K_M	V_{max} mM/min
mM					
15	—	25	5.3	0,23	2,7
25	—	25	1+.2	0 45	3,56
30	—	25	56,8	2,2	3,78
—	0,05	0,15	0,09	0 6	0,5
—	0,1	0 13	0,09	0,7	0.5
—	0.2	0,1	0.09	0,9	0,5

while the role of Co^{2+} most probably consists in the stabilization of the enzyme.

It was established by a special experiment that the stabilizing effect of Co^{2+} ions depends on their concentration and that the maximum effect is reached at a concentration of about 1 mM.

We have previously reported that Str. atratus glucose isomerase possesses a group substrate specificity, i.e., it is capable of isomerizing not only D-glucose but also D-xylose [13]. A further study of this enzyme has shown that Mg^{2+} ions are necessary for the isomerization of both these substrates, while Co^{2+} exhibit a stimulating effect only in association with Mg^{2+} ions, and to a more considerable degree on the isomerization of D-glucose than that of D-xylose. Mn^{2+} ions do not activate the isomerization of either substrate. Similar investigations have been conducted by Danno et al. [7] on the glucose isomerase from B. coagulans, strain HN-68. A distinguishing feature of this enzyme is the fact that its D-xylose-isomerizing activity is Mn^{2+}-dependent [7].

Figures 3 and 4 show graphs of the dependence of the rate of the reaction on the concentrations of Mg^{2+} and Co^{2+} ions in the Lineweaver–Burk coordinates [8]. The calculated corresponding affinity constants are, for Mg^{2+}, K_M = 25 mM and, for Co^{2+}, K_M = 0.15 mM.

The maximum effect was observed under the combined action of Mg^{2+} and Co^{2+} ions. For the further study of this effect we determined the rates of isomerization of D-glucose into D-fructose at various concentrations of Mg^{2+} ions in the absence and in the presence of Co^{2+} ions. The results, treated by the Lineweaver–Burk method, showed that although Co^{2+} ions do not change, the value of K_M for Mg^{2+} ions, they increase the maximum rate of the reaction. These results agree with those obtained for S. albus [9] and Actinomyces olivocinereus [3] and permit the assumption that metal cations do not compete with one another for the same binding section in the enzyme molecule. Consequently, Mg^{2+} and Co^{2+} ions bind

with glucose isomerases in different sections and affect their activities differently. Hence, it is possible that the mechanisms of the activation of the D-glucose-isomerizing reaction with the participation of Mg^{2+} and Co^{2+} ions are different.

Using the Lineweaver–Burk and Dixon method [10], we calculated the kinetic parameters for D-glucose on activation by Mg^{2+} and Co^{2+} ions. The results are given in Table 2 and, according to [3], they show that the value of K_M is independent of the concentration of Mg^{2+} ions. The functional role of these metal ions in the enzymatic reaction is possibly connected with a conformational change of the enzyme molecule caused by them, with the formation of an active ternary complex, Mg^{2+}–E–S. It can be seen from Table 2 that with an increase in the concentration of Mg^{2+} ions the value of K_M increases, which again shows the binding of the Mg^{2+} ions with the enzyme and not with the substrate.

As follows from Table 2, characteristic for Co^{2+} ions is a dependence of K_M on the concentration of this metal with, at the same time, invariability of K_{cat} and V_{max}, which shows the stimulating role of Co^{2+} ions in the binding of the enzyme with glucose. From the values of K_M given, it can be stated that the enzyme has a greater affinity for Co^{2+} ions. The role of Co^{2+} ions in enzymatic isomerization probably consists in the formation of cross-links similar to disulfide bonds within the glucose isomerase molecule and the stabilization in this way of the native enzyme at a high temperature.

EXPERIMENTAL

The conditions for cultivating the strain of Str. atratus have been described previously [11].

We used the highly purified glucose isomerase of which the isolation and purification were described in [12]. Enzymatic activity was determined by a known method [12]. The glucose isomerase of the purified preparation had a specific activity of 41.5 units/mg of protein at a concentration of it of 0.05 mg/ml.

The concentration of D-glucose used for determining activities under the influence of metal ions and the kinetic parameters of this reaction was 0.1 M.

A solution of the enzyme free from metal ions was obtained with the aid of dialysis against 0.005 M phosphate buffer, pH 8.0, containing 0.01 M EDTA for 15 h.

The action of the metal ions on the enzymatic activity was studied by determining it under the same conditions, except that the concentrations of Mg^{2+} and Co^{2+} ions added to the reaction mixture were varied from 0.01 mM to 0.5 M and from 0.01 mM to 10 mM, the other metal ions (Fe^{2+}, Mn^{2+}, Ba^{2+}, Cu^{2+}, Ca^{2+}, Hg^{2+}, Pb^{2+}, Zn^{2+}, and Cd^{2+}) being added in a concentration of 1 mM.

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