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Calorimetric investigations of the enzyme catalyzed sucrose hydrolysis[1]

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Abstract

The study presents the results of calorimetric investigations of the invertase catalyzed sucrose hydrolysis. The aim of the investigation is the kinetic interpretation of calorimetric curves with respect to the inhibitor effect of selected heavy metal ions and the calorimetric determination of the invertase activity.

Keywords: Calorimetry; Enzymatic activity; Heavy-metal ions; Inhibition; Invertase

1. Introduction

In previous studies [1,2], we have reported the results of calorimetric investigations of the inhibitor effect of heavy-metal ions (Cd(II), Zn(II), Pb(II), As(III), As(V)) on the enzyme activity of urease. The obtained results allowed a quantification of the inhibitor effect based on the initial reaction rate of enzyme-catalyzed urea hydrolysis. In comparison to these measurements, we report in this paper investigations of the influence of the same heavy-metal ions on the activity of the enzyme invertase. Moreover, we employ Ag(I)-ions to extend the spectrum of heavy-metal inhibitors. The results of investigation of an enzymatic reaction in the presence of heavy-metal ions show the possibility of the determination of the relative enzymatic activity. Thus, we used the calorimetric measurements for determination of the specific activity of the enzyme invertase.

2. Experimental

The calorimetric measurements were performed in an isoperibolic calorimeter [3]. Previous to the reaction in the calorimeter, the investigated systems were thermostated to a constant temperature of 298.15 K. The parameters for a mathematical model of the used calorimetric arrangement are determined with different calibration experiments. With this model, the experimental temperature–time curve is deconvoluted in order to obtain the corresponding slope for adiabatic conditions. The details of the calorimetric arrangement are given in [4].

A solution, consisting of buffer (0.05 M acetate buffer, pH 4.6), heavy-metal ions and the enzyme invertase EC 3.2.1.26 was placed in the calorimeter cell. The sucrose (Merck) solution was arranged in the ampoule. The reaction was initiated by breaking the ampoule in the calorimeter cell. In order to ensure a defined metal content (Cd(II), As(V), Zn(II)) in the system, aqueous metal standard solutions (Titrisol, Merck) with a content of 1 g/l were used. In the case of As(III), a solution of 1 g/l was produced by dissolving As_2O_3 in distilled water.

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[1]Dedicated to Prof. Günter Marx from the TU-Chemnitz on the occasion of his 60th birthday.

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The photometric determination of the invertase activity was performed with UV/VIS – spectrometer (type: UNICAM 8625). The following enzymes and substances were used for the photometric investigations:

- peroxidase EC 1.11.1.7 from horseradish, Serva;
- glucoseoxidase EC 1.1.3.4 Aspergillus niger, Feinchemie GmbH Sebnitz;
- 2,2′-Azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), Sigma–Aldrich.

The applied photometric determination of the invertase activity consists of two steps. In the first step, sucrose is converted into fructose and glucose catalyzed by the invertase. The glucose produced during a definite time is determined in the second step enzymatically. Glucose is converted by glucoseoxidase into gluconolactone and H_2O_2. The produced H_2O_2 is decomposed by the enzyme peroxidase, and hydrogen is transferred from ABTS (2,2′-azino-bis(3-ethylbenzthiazoline)-6-sulphonic acid). The quantity of transferred hydrogen can be measured by the absorbance of the formed dye at 405 nm.

3. Discussion

Sucrose hydrolysis is catalysed by invertase in the acetate buffer corresponding to

$$\text{sucrose} + H_2O \rightarrow \text{glucose} + \text{fructose}.$$

In accordance with the literature [5], we determined for the sucrose hydrolysis the molar reaction enthalpy $\Delta_R H = -(15.4 \pm 0.1)$ kJ/mol. For the measurements on urea hydrolysis, the value of the molar reaction enthalpy was not affected by heavy-metal ions [1]. The observed value for molar reaction enthalpy of sucrose hydrolysis is also independent of the kind and concentration of investigated inhibitor (see Table 1), indicating a complete extent of reaction.

For the purpose of kinetic evaluation of the adiabatic temperature–time curve from the calorimetric measurement, it was assumed that the temperature change ΔT during time t is proportional to the extent of hydrolysis. By extrapolation of the ΔT vs. t curve for an initial range (t<110 s) to t=0, a value proportional to the initial reaction rate, v_0, is obtained [1]:

$$v_0 = -\left(\frac{dc_s}{dt}\right)_{t=0} = \left(\frac{c_s^0}{\Delta T_{max}}\right)\left(\frac{\partial \Delta T}{\partial t}\right)_{t=0} \quad (1)$$

where c_s^0 is the total sucrose concentration, c_s the sucrose concentration at time t, and ΔT_{max} the maximum value of ΔT (ΔT at complete conversion).

3.1. Inhibitor effect of heavy-metal ions

Experiments were performed to determine the influence of the Ag(I)-ions on the initial reaction rate. Fig. 1 shows the strong reduction of v_0 depending on the concentration of Ag(I)-ions. Even 4 μmol Ag(I)/kg solution may lead to a 50% inhibition of the invertase activity. The limit of thermal detection of Ag(I)-ions is 0.5 μmol Ag(I)/kg solution or 50 ppb, respectively. In addition to Ag(I)-ions, in order to compare with the results from the studies of the urease inhibition by heavy-metal ions, we also investigated the influence of the same heavy-metal ions on enzymatic sucrose hydrolysis. We have chosen a concentration of these heavy-metal ions, which had led to a 50% inhibition of the urease activity. As seen in Table 2, this concentration did not remarkably reduce the initial reaction rate of sucrose hydrolysis. In agreement with the results for the enzymatic urea hydrolysis, As(V)-ions did not inhibit the invertase activity.

The enzymes invertase and urease differ in their structures. It can be assumed that the small number of sulphhydryl groups in the protein molecule of the invertase, which is only indirectly effective for the catalytic process, affects the inhibition by heavy-metal ions. The strong inhibition of Ag(I)-ions may be explained with the possibility of disproportionation reaction of the Ag(I)-ions with RSSR-groups of the protein molecule as described in [6].

Table 1
Effect of some heavy-metal ions on the molar reaction enthalpy of sucrose hydrolysis

Heavy metal	—	Cd(II)	Zn(II)	As(III)	As(V)	Ag(I)
$\Delta_R H$ (kJ/mol)	−15.4±0.1	−15.7 −15.4	−15.3 −15.1	−15.8 −15.9	−15.5 −15.2	−15.4±0.2

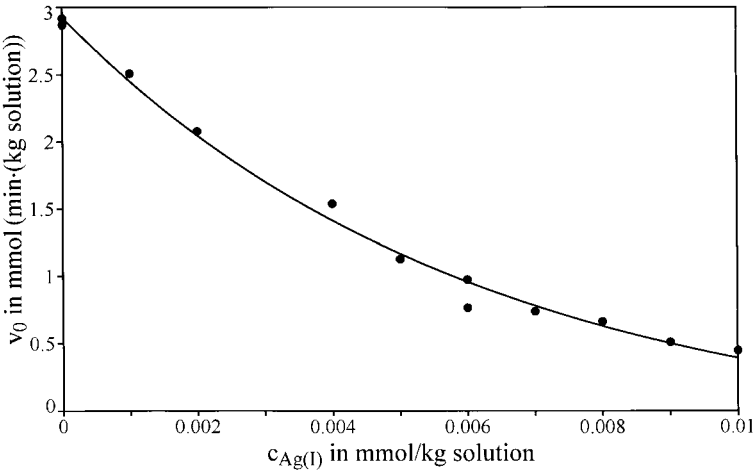

Fig. 1. Dependence of the initial reaction rate on the concentration of Ag(I)-ions.

Table 2
Effect of some heavy-metal ions on the initial reaction rate of the sucrose hydrolysis

Heavy metal	—	Cd(II)	Zn(II)	As(III)	As(V)
c(metal) in mmol/(kg solution)	—	0.33	0.04	0.41	0.48
v_0 mmol/(min(kg solution))	2.72	2.62	2.66	2.63	2.69
	2.68	2.67	2.68	2.77	2.76

3.2. Determination of the specific activity of the invertase

From the experimental results of calorimetric investigations it seems possible to determine the activity of the used enzyme. Compared to the other mostly very enzyme-specific methods, the calorimetric measurements are relatively simple and very easy to apply for the determination of different activities. In the following we describe the calorimetric determination of the specific activity of invertase. The obtained values for the activity are compared with the results from measurements with a well-known photometric method [7].

Taking into consideration Eq. (1) and the Michaelis–Menten relation Eq. Eq. (2), the maximum reaction rate v_{max} can be calculated from the calorimetrically determined rate constant k:

$$v_0 = \frac{v_{max} c_s^0}{K_M + c_s^0} \tag{2}$$

$$k = \frac{1}{\Delta T_{max}} \left(\frac{\partial \Delta T}{\partial t} \right)_{t=0} = \frac{v_{max}}{(K_M + c_s^0)} \tag{3}$$

$$v_{max} = (K_M + c_s^0) k \tag{4}$$

For the calculation of v_{max} according to Eq. (4), we have to determine the Michaelis constant K_M under comparable conditions (buffer system, pH). The Michaelis constant is determined from calorimetric investigations with different sucrose concentrations, varied in the range from 12 to 23 mmol/kg solution. Such measurements provide a value of K_M=46± 2 mmol/kg solution. This result is in agreement with values, in the literature, of K_M=49 mmol/l [8] and of K_M=42 mmol/l [9], which were determined by the use of 0.5 molar acetate buffer with a pH=4.5.

The specific activity of the an enzyme can be calculated from the following equation:

$$\text{specific activity} = \frac{v_{max} m_{solution}}{m_E} \tag{5}$$

where $m_{solution}$ is the mass of the reacting solution in g,

m_E the mass of enzyme in mg, and v_{max} the maximum reaction rate in mmol/(min(kg solution)).

According to Eq. (5), we obtained 225 U/mg for the specific activity of invertase from calorimetric measurements.

A photometric method as described in the experimental part should be suitable to prove the activity value from calorimetric investigations. We obtained the value of specific activity of the invertase at 216±7 (U/mg) by photometry.

The results of the determination of the specific activity by the two methods are in good agreement. The calorimetric determination of the enzyme activity has crucial advantages in contrast to the photometric method, because of the much smaller expenditure.

4. Summary

In this work, the enzymatic sucrose hydrolysis was investigated by calorimetric measurements. The influence of heavy-metal ions on sucrose hydrolysis was examined. Ag(I)-ions proved to be strong inhibitors of the invertase. Even 4 μmol Ag(I)/kg solution led to a 50% inhibition of invertase activity. In contrast to the results obtained for the enzyme urease [2], we have not observed an inhibition of the activity of the enzyme invertase by the other investigated heavy metals. Furthermore, the calorimetric investigations of sucrose hydrolysis are utilized to determine the specific activity for invertase. The results are in good agreement with the photometrically determined values.

Acknowledgements

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