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Biochemical analysis of cyclodextrins using an enzyme thermistor

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Abstract

A thermostable α-amylase catalyzed the exothermal hydrolysis of cyclodextrins. It was immobilized covalently via a spacer on controlled pore glass (CPG-10) or Silicagel. The temperature signal caused by the reaction heat of the cyclodextrin hydrolysis was determined in a one-column calorimetric system (enzyme thermistor). It was correlated to the cyclodextrin concentration and depended on the type of enzyme carrier and kind of cyclodextrin hydrolyzed. The proposed technique offers a direct route to the determination of α-amylase activity, and the results are of importance for analysis of cyclodextrin concentration.

Keywords: Amylase; Biosensor; Calorimetry; Cyclodextrin; Enzyme thermistor

1. Introduction

Enzyme-catalyzed reactions, such as the hydrolysis of cyclodextrins, are coupled to heat production as a rule. The enzyme thermistor principle is based on this fact and measures the temperature difference evolved in a flow unit [1, 2]. Thus, test systems for biochemical analysis of carbohydrates and other compounds were developed [3–5]. At present, no test system is available for calorimetric analysis of cyclodextrins. Cyclodextrins (Schardinger dextrins) are compounds that are useful in many fields of application, such as the separation of fatty acids and the stabilization of pharmaceuticals and fertilizers [6]. They are composed of six to eight glucose units linked by α-1,4-bonds.

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The corresponding cyclodextrins formed are designated as α-, β- and γ-cyclodextrin. Cyclodextrins can be produced by cyclization of starch catalyzed by cyclodextrin glycosyltransferase which is synthesized by several *Bacillus* strains.

Special α-amylases catalyze the hydrolysis of α-1,4-bonds in cyclodextrins accompanied by heat production in the range of −48 up to −53 kJ mol^{-1} [6–8]. For comparison, the hydrolysis of sucrose catalyzed by β-fructofuranosidase is coupled to a reaction heat of −15.25 kJ mol^{-1} which has been sufficient to develop test systems for biochemical analysis of sucrose concentration [4, 7, 9]. Consequently, biochemical analysis of cyclodextrins should also be feasible which is of practical importance in the production and application of cyclodextrins.

2. Methods

2.1. Calorimetric unit

The enzyme thermistor used was a one-column flow system described in detail elsewhere [1, 2], Fig. 1. The temperature difference between the bottom and the top of the column was measured continuously. The column was placed in a thermostatted metal block isolated from the surroundings by polyurethane foam. It was filled with immobilized enzyme preparations on CPG-10 or Silicagel acting as a biocatalyst in the enzyme-catalyzed hydrolysis of cyclodextrins. A heat exchange unit kept the temperature of the influent liquid medium constant (25, 30 or 37°C).

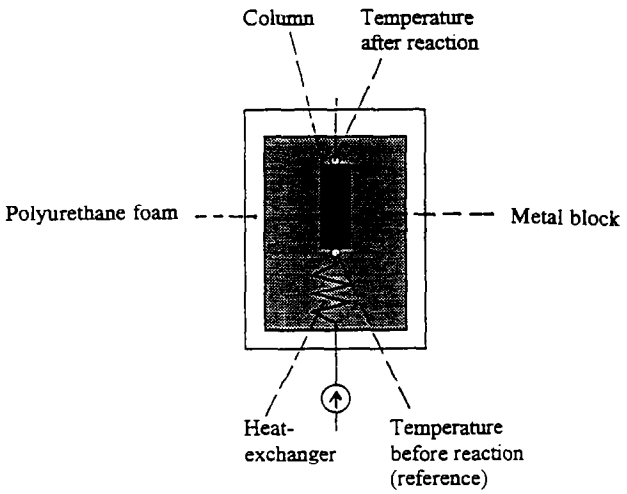

Fig. 1. Schematic drawing of the enzyme thermistor unit.

2.2. Preparation of immobilized enzyme

CPG-10 (Corning, N.Y.) and Silicagel of particle size 40–63 μm (Merck) were used as carriers. The carriers were silanized and activated with glutardialdehyde as described in detail elsewhere [10]. Termamyl (Novo Nordisk), a thermostable neutral α-amylase, was bound covalently to the carrier via the spacer formed from silane and glutardialdehyde. One part of the Termamyl liquid (enzyme activity about 400 U ml^{-1}) was mixed with four parts of 0.1 M sodium phosphate buffer at pH 7. The protein concentration should be preferably 1%. About 1 g of carrier was added to the diluted Termamyl solution (0.02 dm^3) and incubated for four hours at room temperature with gentle mixing. Afterwards, the enzyme preparations were poured into the sample column of the enzyme thermistor ready for hydrolysis of cyclodextrin in influent cyclodextrin solutions.

2.3. Biochemical analysis of α- and β-cyclodextrin

α- and β-Cyclodextrin solutions with concentrations between 0.1 and 10 mmol dm^{-3} were pumped through the column of the enzyme thermistor. The optimum flow rate was in the range 0.03–0.12 $dm^3\ h^{-1}$. The exothermal biochemical reactions occurring were as follows [7,8]:

α-Cyclodextrin (cyclohexaamylose)

$$\text{cyclohexaamylose(aq)} + 6H_2O \xrightarrow{\alpha\text{-amylase}} 6\,\text{D-glucose(aq)}$$

$$Q = -53.5\ \text{kJ mol}^{-1}$$

β-Cyclodextrin (cyloheptaamylose)

$$\text{cycloheptaamylose(aq)} + 7H_2O \xrightarrow{\alpha\text{-amylase}} 7\,\text{D-glucose(aq)}$$

$$Q = -48\ \text{kJ mol}^{-1}$$

3. Results and discussion

The dependence of the temperature signal (enzyme thermistor) on the β-cyclodextrin concentration is shown in Figs. 2a and b. In these experiments, enzyme preparations on CPG-10 were used. The β-cyclodextrin concentration increased with the temperature difference and with it the reaction heat in a concentration range of 0.1 up to 10 mmol dm^{-3}. At higher β-cyclodextrin concentrations, a saturation effect occurred and a maximum temperature signal of 0.08883 K was approximated.

Further results were obtained with enzyme preparations on Silicagel, see Fig. 3 and Table 1. α- and β-Cyclodextrin solutions were investigated experimentally. The measured temperature signal was proportional to the concentration of α-cyclodextrin in a concentration range of 1–10 mmol dm^{-3}. In contrast to studies of α-cyclodextrin solutions, β-cyclodextrin hydrolysis was coupled to an increased temperature signal

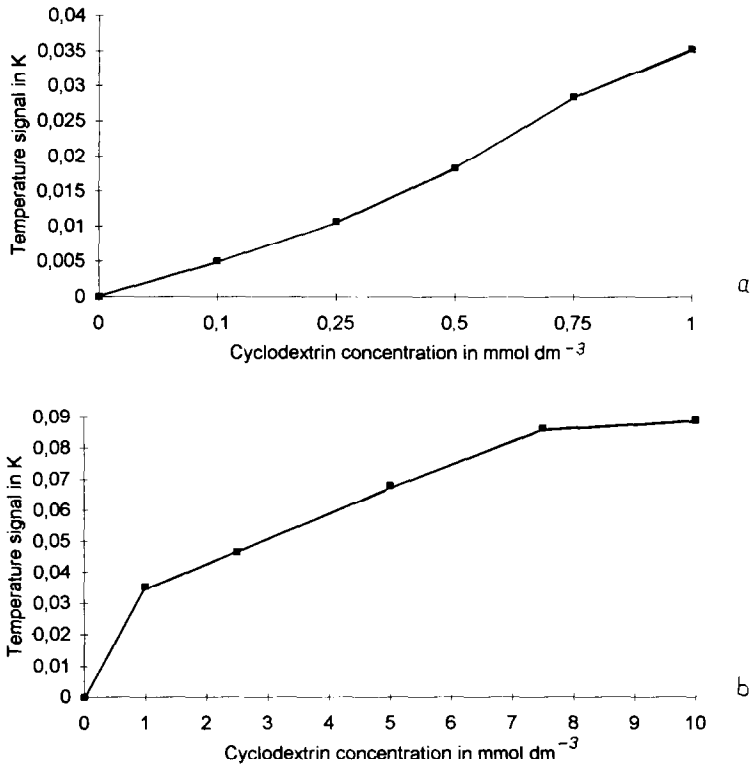

Fig. 2. Dependence of temperature signal (enzyme thermistor) on β-cylodextrin concentration at 32°C and pH 6.5 (Termamyl on the activated carrier CPG-10): (a) cyclodextrin concentration range 0.1–1 mmol dm^{-3}; (b) cyclodextrin concentration range 1–10 mmol dm^{-3}.

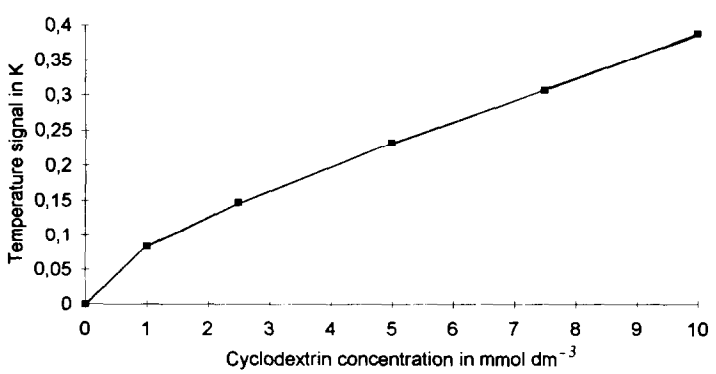

Fig. 3. Dependence of temperature signal (enzyme thermistor) on α-cylodextrin concentration at 32°C and pH 6.5 (Termamyl on the activated carrier Silicagel, pore size 40–63 μm).

Table 1
Temperature signal of immobilized α- and β-cyclodextrin (cyclodextrin concentration, 10 mmol dm^{-3}; carrier, Silicagel of pore size 40–63 μm)

Sample	Temperature signal/K
α-Cyclodextrin	0.3973
α-Cyclodextrin	0.3747
β-Cyclodextrin	0.4983
β-Cyclodextrin	0.4944

and also to the reaction heat, Table 1. These results contradict studies of Takahashi and Ono [8] who detected a higher reaction heat in the case of α-cyclodextrin hydrolysis compared to the values of β-cyclodextrin hydrolysis in aqueous solution at pH 5.0 (0.02 molar acetate buffer). However, the results determined by enzyme thermistor measurements seem to correlate with the number of glucose units in α- and β-cyclodextrins:

α-Cyclodextrin 10 mmol dm^{-3} Experimental peak height, 32000 Experimental temperature difference, 0.03865 K

β-Cyclodextrin 10 mmol dm^{-3} Experimental peak height, 41000 Experimental temperature difference, 0.04952 K Expected temperature difference, 0.04509 K

The expected value of β-cyclodextrin hydrolysis was calculated from the experimental temperature difference of α-cyclodextrin hydrolysis; α-cyclodextrin includes six α-1,4-bonds between glucose units. The deviation of the experimental and expected values can be explained by the different ring tension of α- and β-cyclodextrins.

4. Conclusions

Biocalorimetry is a useful method for the biochemical analysis of α- and β-cyclodextrin because the heat evolution of cyclodextrin hydrolysis is increased compared to those of hydrolysis of other carbohydrates, such as lactose or sucrose. A suitable instrument for these investigations is the enzyme thermistor which is a calorimetric flow system. Termamyl, a thermostable α-amylase, immobilized on activated CPG-10 or Silicagel, was used as the biocatalyst in the experiments. The analysis of cyclodextrin concentration was carried out in a concentration range of 0.1–10 mmol dm^{-3}. Further experiments should concentrate on investigations of enzyme-catalyzed hydrolysis of α-, β- and γ-cyclodextrin in aqueous solution using an isothermal flow microcalorimeter with a mixing vessel, and on statistical evaluation of the results described above by further experiments using the enzyme thermistor.

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