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GELATIN-ENTRAPPED WHOLE-CELL INVERTASE

Palma Parascandola & V. Scardi*

Cattedra di Chimica delle Fermentazioni e Batteriologia Industriale, Facoltà di Scienze, Università di Napoli, via Mezzocannone 8, 80134 Napoli, Italy

SUMMARY

The investigated biocatalyst consists of gelatin-entrapped cells of Saccharomyces cerevisiae retaining invertase activity. Comparative examination of pH profile, apparent K_m, saturation velocity and activation energy indicates that the entrapment procedure did not influence invertase affinity with sucrose but lead to some loss of activity probably due to either enzyme inactivation or cell-wall impairment as well as to substrate diffusion limitation in the gel matrix.

INTRODUCTION

The entrapment of yeast cells (Saccharomyces cerevisiae) in gelatin by the method described previously (Gianfreda et al., 1980) yields preparations with fairly good retention of invertase activity. It has been demonstrated that the gelatin-entrapped yeast cells are unable to ferment either sucrose or glucose, though they exhibit a more stable invertase activity as compared with the intact cells (Parascandola & Scardi, unpublished results). Thus, gelatin-entrapped yeast cells represent an inexpensive, stable and convenient form of immobilized invertase particularly suitable for continuous hydrolysis of sucrose.

The present paper describes the results of an investigation into the kinetic properties of such a whole-cell invertase preparation, which is potentially useful for the production of invert sugar.

EXPERIMENTAL

Freeze-dried baker's yeast was entrapped in gelatin according to the method of Gianfreda et al.(1980). A 35 mesh granulated preparation with a cells-to-gelatin of 1:10 (on a dry wt basis) was used throughout all the experiments. Yeast invertase and sucrose were from Boehringer Biochimia (Milan, Italy) and Serva Feinbiochemica (Heidelberg, Germany), respectively.

Two types of reactors were used to determine the reaction rates of the free invertase, whole-cell invertase and gelatin-entrapped whole-cell invertase. A batch reactor for sucrose hydrolysis by the free enzyme and whole-cell enzyme was a jacketed glass vessel (70 ml) equipped with a magnetic stirrer. During the course of the reaction, six samples were pipetted out at fixed time-intervals and assayed for reducing sugars. As the total volume of the samples did not exceed 1.0 percent of that of the reaction's, the enzymic concentration was taken to be constant during the course of the reaction. A small packed-bed reactor consisting of a plastic column (5 x 70 mm), with adjustable ends (to control the bed volume) and water jacket added, was used for continuous sucrose hydrolysis by the gelatin-entrapped whole-cell invertase. Substrate solutions were fed to the column at a constant flow rate (0.3 ml/min) by a peristaltic pump; before entering the column, solutions were brought to the temperature of the column by passing them through a preheating coil. The reaction rate was evaluated by measuring the concentration of reducing

sugars in the effluent when a steady-state was established, generally after an outflow of about 10 ml (i.e. about 20 reactor volumes). After each run the packed gel particles were washed by pumping deionized water or buffer solution; the washings were collected to check possible cell leakage from gel particles. To eliminate change in the flow rate by excessive swelling or shrinking, especially in consequence of modified experimental conditions (pH, temperature), gelatin particles were mixed with 60 mesh glass beads (1:3, by volume) before packing them into the column.

Reducing sugars were measured by the method of Nelson (1944), with glucose as a standard. Reaction rate was expressed as μmoles of sucrose hydrolysed/min under the conditions of the experiment.

RESULTS AND DISCUSSION

The effect of pH on the activity of the gelatin-entrapped whole-cell invertase was investigated at 30°C in acetate buffer at a constant ionic strength of 0.1 and compared with that of the whole-cell enzyme and free enzyme (Fig. 1). The optimum pH of the entrapped whole-cell invertase was found to be 4.65, as though the optimum pH of the whole-cell enzyme had been shifted of about 0.2 pH unit towards the alkaline values upon entrapment in gelatin. Besides this negligible shift, the pH/activity profile of the entrapped whole-cell invertase appeared as a normal bell-shaped curve, whereas that of both whole-cell enzyme and free enzyme was an asymmetrical curve with broader alkaline limb (Fig. 1). Thus, a more pronounced decrease in the activity of the gelatin-entrapped whole-cell invertase is to be expected when pH increases above 4.65; however, this response to pH change is scarsely significant because only neutral products are formed on sucrose hydrolysis.

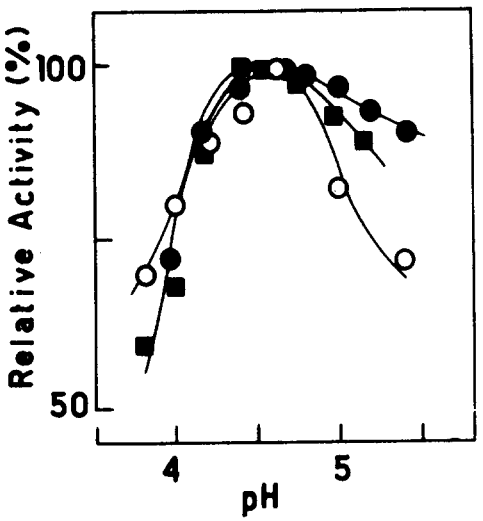

Fig. 1. Effect of pH on invertase activity.

(●) Free enzyme; (■) whole cell enzyme; (○) gelatin-entrapped whole-cell enzyme.

All experiments were carried out at 30°C in acetate buffer (I = 0.1). Highest enzyme activity was taken as 100.

Using various concentrations of sucrose between 7.0 and 100 mM, the rates of batch sucrose hydrolysis by whole-cell invertase (yeast cells conc. 14.5 µg dry wt of cells/ml) and continuous sucrose hydrolysis by gelatin-entrapped whole-cell invertase (packed-bed containing 4.74 mg dry wt of yeast cells) were determined at 30°C at pH 4.65. From Lineweaver & Burk plots the following values of half-saturation constant (apparent K_m) and saturation velocity per mg dry wt of yeast cells (V'_s) were calculated: 30 mM and 4.38 µmol/min per mg for the whole-cell invertase; 50 mM and 1.36 µmol/min per mg for the gelatin-entrapped whole-cell invertase. However, if the packed-bed reactor used in continuous sucrose hydrolysis was assumed to behave as an ideal plug-flow reactor, an apparent K_m of 36.4 mM was calculated from the slope of the straight line obtained by plotting $S_o X$ against $\ln(1 - X)$, where X, the fraction of sucrose hydrolysed in the column, is given by $(S_o - S)/S_o$, S_o and S being the sucrose concentration in the feed and effluent, respectively. Since the apparent K_m determined by this plotting technique in general decreases with increasing flow rate, it cannot be excluded that at flow rates higher than those used in these experi-

ments (0.3 ml/min) the apparent K_m of the gelatin-entrapped whole-cell invertase may approach the K_m of the whole-cell invertase in stirred suspension. In practice, no significant change in the affinity of active invertase with sucrose seemed to occur on entrapment of whole yeast cells in gelatin.

It is interesting to note that the K_m values of yeast invertase in free solution, 16.7 mM as determined by Cantarella et al. (1977), is about half the value of the apparent K_m for both entrapped and unentrapped whole-cell invertase; similarly, an approximate two-fold increase in the K_m of yeast invertase was also observed when the enzyme was chemically attached to an insoluble support (Filippusson & Hornby, 1970; Usami et al., 1971). In other words, whole-cell invertase seems to behave as a naturally occurring immobilized enzyme, in agreement with what is thought to be the molecular anatomy of the cell wall of Sacch. cerevisiae: invertase - a glycoprotein enzyme normally located in the wall structure of this yeast - has been associated with the mannan-proteins (proteins covalently bound to mannan) in the mannan layer.

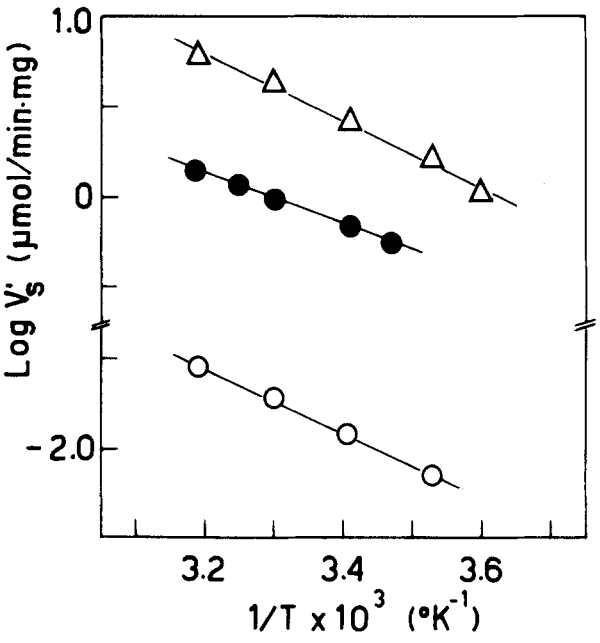

Fig. 2. Effect of temperature on invertase activity. (△) Whole-cell enzyme; (○), (●) gelatin-entrapped whole-cell enzyme in batch reactor and packed-bed reactor, respectively.

Reactions were carried out in acetate buffer (0.1 I, pH 4.65).

The saturation velocity per mg dry wt of yeast cells (V'_s) for the gelatin-entrapped whole-cell invertase was about 30 percent of that for the unentrapped whole-cell enzyme. This decrease in activity may be ascribed to either inactivation of enzyme or impairment of cell-wall structure as well as to internal diffusion limitation of substrate. In an attempt to elucidate this point, sucrose hydrolysis by free invertase, whole-cell invertase and gelatin-entrapped whole-cell invertase (also tested in stirred suspension) was performed in acetate buffer (0.1 I, pH 4.75) at temperatures ranging from 5 to 45 °C and the corresponding values of V'_s were calculated. From the Arrhenius plots of ln V'_s (Fig. 2) the following values of the activation energy were obtained: 9.1 and 8.7 kcal/mol for the free enzyme (not shown in Fig. 2) and whole-cell enzyme, respectively; 8.1 and 6.6 kcal/mol for the gelatin-entrapped whole-cell enzyme in batch and packed-bed reactor, respectively. Whether the decrease in activity is attributable or not also to reagents used for entrapping yeast cells, further research in this direction is necessary. In addition, experiments should be also performed to optimize operational conditions for sucrose hydrolysis by gelatin-entrapped whole-cell invertase (particle size, substrate flow rate,etc).

ACKNOWLEDGEMENT

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