PREPARATION AND CHARACTERIZATION OF MICROBIAL GLUCOAMYLASE IMMOBILIZED IN METHYLTRIETHOXYSILANE/TETRAETHOXYSILANE SOL-GEL MATRICES

OBȚINEREA ȘI CARACTERIZAREA GLUCOAMILAZELOR MICROBIENE IMOBILIZATE ÎN MATRICI SOL-GEL

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Immobilization of biocatalysts helps in their economic reuse and stabilizes the enzymes structure, thereby allowing their applications even under harsh environmental conditions of pH, temperature and organic solvents. The supports and the immobilization method are important for the immobilization efficiency. Amylases held the maximum market share of enzyme sales with their major applications ranging from food, fermentation, textile and paper industries. The aim of this work was to implement a simple and efficient method for the entrapment of microbial glucoamylase in a silica matrix, by the sol-gel method, using different alkoxysilanes as gel precursors. The performance of the enzyme immobilized on silica supports was investigated and compared with that of the free one. The immobilization conditions were varied, and their effect on the performance of the immobilized enzyme was analyzed with reference to their biochemical and kinetical properties.

Key Words: glucoamylase, inorganic materials, sol-gel immobilization, kinetic parameters.

Introduction

In order to improve the performance of the biocatalyst, it is preferably to find an easily handled immobilization method and also a cheap, stable material that can maintain high biological activity of the enzyme. In recent years, sol-gel derived materials have appeared as a new technique for the immobilization of biomolecules [1, 2]. The principal advantages for the sol-gel process are the room temperature processing conditions, chemical inertness, low biodegradation, negligible swelling effects, tunable porosity, the ease with which the microstructure of the material can be modified by varying the process parameters, and high purity of sol-gel derived glasses which make them ideal for many applications, especially for biosensors [3].
Amyloglucosidase [1, 4-α-D-glucan glucohydrolase (E.C.3.2.1.3)] is one of the most economically important enzymes used in many industrial processes [4]. The aim of this work was to implement a simple and efficient method, namely the sol-gel method, for the entrapment of microbial glucoamylase in a silica matrix, using methyltriethoxysilane (MTES) and tetraethoxysilane (TEOS) in different molar ratios, as gel precursors. The performance of the enzyme immobilized on silica supports was investigated and compared with that of the free one. The performance of the immobilized enzyme was analyzed with reference to its biochemical and kinetical properties.

Materials and Methods

Glucoamylase (AMG) from Aspergillus niger was obtained from Novo. The precursors used for sols preparation, tetraethoxysilane (TEOS) and methyltriethoxysilane (MTES), were from Aldrich. Soluble Žulkowski starch was purchased from Merck. All other chemicals were of analytical grade and were used without further purification.

Hybrid matrices were prepared using as Si-precursors tetraethoxysilane (TEOS) and methyltriethoxysilane (MTES). The mixture containing the alkoxides in different molar ratios (MTES:TEOS 1:1, 2:1 and 3:1, respectively) and enzymatic solution, containing 0.075 mL AMG, (1:1.72, v/v) were mixed with PVA 22.000 (polyvinyl alcohol 22.000) 4%, NaF 1 M and isopropyl alcohol (2:1:1, v/v) [5].

Reducing sugars assay: 0.5 mL soluble starch (1%), 0.4 mL citrate-phosphate buffer (0.15 M, pH 4.6) and 0.05 g immobilized biocatalyst were kept for 5 min. at 25°C. 1 mL 3,5-dinitrosalicylic acid (DNS, 1%) was added. The samples were boiled in water 10 min. and 10 mL water was added. The samples were filtered and assayed at 540 nm against blank containing soluble starch, citrate-phosphate buffer, 3,5-dinitrosalicylic acid and distilled water. One unit of AMG activity was defined as the amount of enzyme required to produce 1 μmol glucose in 5 min at 25°C [6].

Determination of kinetics parameters of native and immobilized enzyme: K_M and V_max were determined by measuring initial rates of Zulkowski starch hydrolysis. Kinetics studies were conducted in citrate-phosphate buffer 0.15 M, pH 4.6, at 37°C in a 50 mL stirred jacketed batch reactor. The starch concentrations were 1.5 - 6.25 mg/mL. The reaction was started by addition of the enzyme (4 mL native enzyme and 40 mg immobilized enzyme) and 1 mL samples were collected every 2 minutes during the first 20 minutes of the reaction. The reducing sugars assay was used for analyze the samples.

Results and Discussions

The dependence of the native and immobilized enzyme activities on temperature was investigated in a range of 20-90°C. The optimal temperature was
60°C for the native enzyme and remained the same for the enzyme entrapped in MTES:TEOS 1:1 and 2:1 matrixes. Only in the case of MTES:TEOS 3:1 matrix it was shifted towards smaller values with approximately 15°C.

We investigated the effect of the pH in the range of 2.6 – 8, at room temperature. As a consequence of the immobilization process, the optimal pH of the entrapped enzymes was shifted towards the acid side (with approximately one pH unit) when compared with that of the native enzyme.

The saturation curves of the initial rates of amylase obtained from experimental data are presented in Fig. 3. The Michaelis-Menten constants $K_M$ and $V_{max}$ for the native enzyme were 1.37 mg/mL and 0.25 μmol glucose/mL·min. The immobilization process affects both the $K_M$ and the $V_{max}$ values. The $V_{max}/K_M$ ratio shows that the catalytic efficiency is 1.5 times enhanced by immobilization in MTES:TEOS 3:1 matrices (Table 1).
Fig. 3 Initial rates (v) vs. substrate concentration ([S]) plots of native and immobilized glucoamylase

Table 1

<table>
<thead>
<tr>
<th>Matrix</th>
<th>$V_{max}$ $\mu$mol glucose/mL·min</th>
<th>$K_M$ mg/mL</th>
<th>$V_{max} \cdot 100/K_M$ $\mu$mol glucose/mg starch·min</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>0.25</td>
<td>1.37</td>
<td>18.24</td>
</tr>
<tr>
<td>MTES:TEOS 1:1</td>
<td>0.094</td>
<td>2.19</td>
<td>4.29</td>
</tr>
<tr>
<td>MTES:TEOS 2:1</td>
<td>0.73</td>
<td>14.47</td>
<td>5.04</td>
</tr>
<tr>
<td>MTES:TEOS 3:1</td>
<td>0.56</td>
<td>2.14</td>
<td>26.17</td>
</tr>
</tbody>
</table>

Conclusions

Optimum pH of immobilized enzyme was shifted to acidic side by approximately one unit when compared to native enzyme optimum pH (4.6).

Optimum temperature was maintained the same for the enzyme immobilized in MTES:TEOS 1:1 and 2:1, decreased by 15°C in the case of MTES:TEOS 3:1, compared to native enzyme (60°C).

The matrixes obtained by the sol-gel method, using MTES and TEOS as precursors, do not have a denaturing effect on the enzyme. The maintaining of the
same optimal pH and temperature as the native enzyme prove it. Moreover the immobilized enzyme can be used in the same pH and temperature conditions as the native counterpart.

The $V_{\text{max}}/K_M$ ratio, which shows the catalytic efficiency, indicate MTES:TEOS 3:1 as the optimum matrix for amyloglucosidase immobilization.

The results of this work show that biomaterials obtained from a mixture of MTES and TEOS, by the sol-gel method, are suitable for many biotransformations allowing the entrapped glucoamylase to retain its whole biological activity.

The sol-gel technology is both simple and inexpensive. The immobilization technique is of low-cost creating the possibility of employing it to other enzyme systems.

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Bibliography


Imobilizarea biocatalizatorilor are o deosebită importanță economică permitând reutilizarea și stabilizarea enzimelor care astfel pot fi folosite în condiții extreme de pH, temperatură, solvenți organici. Atât suportul cât și metoda de imobilizare joacă un rol decisiv în eficiența imobilizării. Amilazele ocupă un loc central pe piața enzimelor fiind frecvent folosite în biotehnologii cu aplicații numeroase și variate în industria alimentară, textilă, a etanolului și a hârtiei. Scopul acestei lucrări a fost aplicarea unei metode simple și eficiente de imobilizare a glucoamilazelor microbiene în matrice de silice, prin metoda sol-gel, folosind diferiții alcoxisilani ca precursorsi. Caracteristicile enzimei imobilizate au fost studiate și comparate cu cele ale enzimei native. Enzima a fost imobilizată în diferite condiții, iar efectul acestora asupra performanței biocatalizatorului a fost studiat prin prisma proprietăților biochimice și cinetice.

Cuvinte cheie: glucoamilază, imobilizare sol-gel, materiale anorganice, parametri cinetici.