Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 52–53 (2008) 189–193
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Nucleoside synthesis using a novel macroporous grafted polyethylene as biocatalyst support

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Available online 20 February 2008

Abstract

Nucleoside analogue synthesis has received much attention because of the wide range of applications that these molecules offer. They are extensively used as antiviral, antitumor and more recently, as starting materials for functional oligonucleotides. Microbial whole cells are efficient, ecological and low cost biocatalysts that have been successfully applied to the preparation of these compounds. A new support for cell immobilization that involves the use of a macroporous polyethylene polymer grafted with chains of polyglycidyl methacrylate–ethylendiamine is described in this paper. High stability and productivity and easy handling are some of the advantages of the here developed biocatalyst.
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Keywords: Polyethylene; Whole cell immobilization; Adenosine; *Escherichia coli* BL21; Nucleoside phosphorylases

1. Introduction

Biocatalysed synthesis of nucleoside analogues is a challenging goal due to the wide spectrum of applications that these molecules display, such as antiviral and antitumor agents and starting materials for antisense oligonucleotides [1,2].

The enzymatic synthesis of modified purine nucleosides through a transglycosylation reaction catalysed by nucleoside phosphorylases provides several advantages over chemical routes, such as regio and stereoselectivity and environmentally clean one pot reactions [3,4]. Moreover, microbial whole cells can be directly used as biocatalysts, what provides a simpler and cheaper methodology since enzyme isolation and purification are avoided [5,6]. Very few reports have so far dealt with the use of immobilized microbial cells for nucleoside synthesis and most of them involved entrapment techniques [7–10]. Although some materials have been successfully used for this last purpose, these polymers have drawbacks such as poor mechanical strength and durability (agar, agarose, alginate, chitosan) or toxicity to microorganisms (polyacrylamide, polyurethane) [11]. Therefore, the search of alternative techniques such as adsorption, may provide new materials for whole cell supports.

Recent advances in macromolecular biomaterial technology combine the effort of scientists in various fields to obtain polymers with well-defined structures and specific chemical, physicochemical, mechanical and biological properties [12]. Due to the fact that microbial cells have predominantly negative charges on their surfaces, they can be efficiently adsorbed on a polymeric material carrying cationic groups. These types of polymers could be obtained by crosslinking, like styrene–divinylbenzene copolymer crosslinked with poly(ethyleneimine) [13] or by radiation-induced graft polymerization (RIGP) [14] like the polymer used in this work. In this last case, amino groups are attached on the grafted polymer branches that form a flexible brush-type structure, which enables their interaction with microbial cells. The main advantage is that while crosslinking reaction is performed in presence of cells, the grafted-type materials are made before cell immobilization takes place and therefore, cell viability is not impaired. This technique allows the preparation of polymeric material on a variety of shapes such as films, fibers, hallow fibers or nonwoven fabric [15] and variation in grafting degree can easily alter the charge density. Besides, their manipulation is more suitable than that of gel beads obtained with the majority of the entrapment supports.

The objective of the present work was to study the behavior of whole cell immobilized onto a novel porous polymeric support in the synthesis of purine nucleosides. The used reaction model was

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doi:10.1016/j.molcatb.2007.12.014

Nomenclature

RIGP	Radiation-induced graft polymerization
PE	Polyethylene
GMA	Glycidyl methacrylated
EDA	Ethylendiamine
GA	Glutaraldehyde

the synthesis of adenosine from uridine and adenine biocatalysed by *Escherichia coli* BL21, which characteristics are fully known in our research group [16,17].

2. Experimental

2.1. Materials

All employed chemicals were of analytical grade. Nucleosides and bases were purchased from Sigma or ICN. Culture media chemicals were from Merck and HPLC grade methanol was from Fischer.

2.2. Support

Macroporous sheets of high-density polyethylene (PE) were kindly donated by Porex Technology (Fairburn, USA). These sheets (1.5 mm wide) have pore volume ranging from 40 to 50% and pore size between 45 and 90 μm. Small pieces of PE were grafted using glycidyl methacrylate (GMA) as described elsewhere [18,19]. GMA-grafted material was reacted with ethylendiamine (EDA) by soaking the grafted material in ethylendiamine:water (1:1, v/v) at 60 °C for 4 h.

2.3. Cell growth and immobilization conditions

E. coli BL21 (ATCC 47092) was grown at 37 °C for 16 h with shaking in 250 ml Erlenmeyer flasks containing 50 ml of LB culture medium: 1% (w/v) meat extract, 0.5% (w/v) yeast extract and 0.5% (w/v) NaCl in deionized water adjusted to pH 7 with KOH. Cells were harvested by centrifugation for 10 min at 12,000 × *g*, washed once with 30 mM potassium phosphate buffer (pH 7) and re-centrifuged. The wet cell paste suspended in 3 ml of buffer was directly incubated with the support during 24 h at 30 °C and with orbital shaking at 200 rpm. The biocatalyst was washed and stored in buffer until use (catalyst load $50{,}000 \times 10^6$ cells/g).

2.4. Synthesis of adenosine (standard conditions)

The standard reaction mixture comprising: 0.058 g biocatalyst prepared as above, 30 mM uridine, 10 mM adenine and 2.5 ml 30 mM potassium phosphate buffer (pH 7), was stirred at 200 rpm at 60 °C for 3 h. Samples were centrifuged at 10,000 × *g* for 30 s and the supernatants were analyzed by HPLC.

2.5. Biocatalyst reuse

After 3 h reaction, the biocatalyst was taken off from the reaction, washed with phosphate buffer and used as biocatalyst for a new biotransformation as indicated above.

2.6. Support reuse

Deactivated biocatalyst was steam sterilized and then treated with aqueous solution of 0.5 N NaOH at 50 °C during 1 h with stirring. The cell-free support was then washed with buffer phosphate and subjected to a new immobilization procedure. The efficiency of the reused support was analyzed carrying out the protocol described in the standard conditions for adenosine synthesis.

2.7. Analysis of reaction products

For quantitative analysis an HPLC equipped with an UV detector (254 nm) and a Kromasil 100 C-18, 5 μm, 25 mm × 0.4 mm column was used. Production of adenosine was determined using as the mobile phase: (1) 6 min water/methanol (95:5, v/v), (2) 3 min gradient to water/methanol (90:10, v/v), (3) 6 min water/methanol (90:10, v/v); and as flow rate: 0.9 ml/min.

2.8. Electron microscopy

Polymeric support loaded with *E. coli* BL21 was socked in fixative solution (4% (w/v) paraformaldehyde, 0.25% (v/v) glutaraldehyde in 0.1 M phosphate buffer pH 7.4) at 4 °C. After 4 h the material was washed with distilled water. Afterward, sample was postfixed for 1 h in OsO_4 (1%, v/v), contrasted with uranyl acetate (1%, v/v), dehydrated and embedded in Durcupan (Fluka Chemic AG). Ultrathin sections were cut from cross-sectional areas and examined and photographed on a Siemens Elmiskop I electron microscope.

3. Results and discussion

3.1. Support characterization

Macroporous polyethylene (PE) was used as the trunk polymer. A vinyl monomer containing an epoxy group, GMA, was grafted onto the PE material and then, the GMA-grafted material was reacted with ethylendiamine as described by Lee et al. [20] giving primary and secondary amino groups.

The shallow area for cell immobilization was 0.1 m^2/g, calculated from nitrogen adsorption/desorption isotherms using a BET-sorptometer. Considering that the microbial area is 2 μm^2, in theory $50{,}000 \times 10^6$ cells/g could be immobilized. Experimentally, the biocatalyst was obtained by shaking a suspension of cells in buffer with the polymer. Different quantities of cells were immobilized (Table 1), observing that similar yields were obtained when 50,000 or $100{,}000 \times 10^6$ cells/g were used. Poorer results were obtained with lower or higher biocatalyst amounts. The last case probably involves diffusion problems related to cell multilayer.

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