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ARTICLE

Hydrolytic and chromatographic studies on the PEGylation of dextranase from *Penicillium* sp.

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Abstract Dextranases catalyze the hydrolysis of the α-l,6-glucosidic bond of the polysaccharide dextran. Dextranases have been isolated from bacteria, yeast and fungi. Purified dextranase enzyme from *Penicillium* sp. was PEGylated (polyethylene glycol modification) with mPEG (5000 Da) and showed an increase in the dextranase protein molecular weight as estimated by Superose 12 (23 ml) column and this increment in the molecular weight is directly proportional to mPEG (5000 Da) concentration until a complete dextranase enzyme PEGylation (disappearance of dextranase peak). The residual activity of partially PEGylated dextranase (mPEG 5000 of 5.8 mg/ml) was 33.8% and for the completely PEGylated dextranase (mPEG 5000 of 29 mg/ml) it was 25.75%. Dextranase PEGylated with mPEG (30,000 Da) showed a little PEGylation at mPEG concentration of 5.8 mg/ml but at a concentration of 29 mg/ml several PEGylated peaks were produced with a difference in dextranase activity toward dextran T500, retardation in the activity with the increasing in the molecular weight was clearly appeared with Sephadex G75 but for Sephadex G200 a little retardation than Sephadex G75 has been appeared.

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1. Introduction

Dextranase (EC 3.2.1.11 α-D-glucan-6-glucanohydrolase) is an enzyme capable of hydrolyzing the α-1,6-glucosidic bond of dextran producing either glucose or isomaltose (endo-dextranase), or isomalto-oligosaccharides (exo-dextranase) depending on the enzyme source [1]. Dextranase plays an important role in sugar industry by reducing dextran formation, dental plaque treatment and production of low molecular weight clinical dextran [2–4].

PEGylation is a procedure of increasing interest for enhancing the therapeutic and biotechnological potential of peptides and proteins. When polyethylene glycol (PEG) is properly

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linked to a polypeptide, it modifies many of its features while the main biological functions, such as enzymatic activity or receptor recognition, may be maintained. PEG conjugation masks the protein's surface and enlarges the molecular size of the polypeptide, thus reducing its renal ultrafiltration, preventing the approach of antibodies or antigen processing cells and reducing the degradation by proteolytic enzymes [5]. PEG has also approved to be used as a vehicle or base in foods, cosmetics and pharmaceuticals, including injectable, rectal topical and nasal formulations. PEG shows little toxicity, and is eliminated from the body intact by either the kidneys (for PEGs < 30 kDa) or in the feces (for PEGs > 20 kDa) [6]. Mumtaz and Bachhawat [7] modified dextranase enzyme with PEG and used liposomes as carrier for delivering both free and PEG-modified dextranase to the liver of mice at similar rate and they found that the PEG-dextranase showed a greater intracellular stability as compared with the native enzyme. The PEG-dextranase could not only degrade the accumulated FITC-dextran but could also prevent further accumulation over a period. The low molecular mass endoglucanase from *Fusarium oxysporium* was chemically modified by two distinct types of amino acid specific modifiers, cyanuric chloride activated polyethylene glycol (CC-PEG) and polyethylene glycol succinimidyl succinate active ester (SS-PEG) specific for lysine attachment and maleimide polyethylene glycol (Mal-PEG) specific for cysteine attachment. Almost total activity loss occurred in the case of enzyme reaction with CC-PEG. In contrast there was no inactivation after enzyme reaction with SS-PEG and Mal-PEG. The modified endoglucanase showed remarkably enhanced stability against alkaline pH. It was also found that the modified enzyme cleaved preferably the internal glucosidic bonds of cellotetraose, cellopentaose and CMC. Thus the modified enzyme retains the endocharacter of the native enzyme [8]. L-asparaginase, an important enzyme used in leukemia treatment, was modified through polyethylene glycol (PEG) conjugation in order to reduce the hypersensitivity reactions caused by the enzyme. The PEGylated enzyme exhibited less immunogenicity with much longer half-time of plasmatic life [9]. This research is undertaken with the objective of studying the dextranase PEGylation with specific reference to its chromatographic and the hydrolytic properties.

2. Materials and methods

2.1. Materials and equipments

Purified dextranase enzyme from *Penicillium* sp. was used as the PEGylated substrate. mPEG-SPA (Mwt. of 5000 and 30,000 Da) was purchased from Nektar™. Superose 12 HR10/30 column (Pharmacia Biotech.); Superose 6 HR10/30 column; Superose 12 prep. grade column (100 ml); Dextran 5 of average Mwt., 5150; Dextran 10 of average Mwt.,10450, Dextran 20 of average Mwt., 21450; Dextran 70 of average Mwt., 70300; Dextran 150 average Mwt.,147550; Dextran T250 of average Mwt., 238200 and dextran T500 were Pharmacia biotechnology products. Amicon Ultrafiltration cell using Amicon Millipore membrane of 10 pk was also used to concentrate enzyme samples. All chromatographic processes were carried on ÄKTA FPLC (Amershampharmacia Biotech.).

2.2. Dextranase assay and protein measurement

Dextranase enzyme activity was measured by incubating the enzyme with buffered dextran solution (2% dextran T250 dissolved in 0.05 M acetate buffer pH5) for 10 min at 55 °C [10,11]. The activity was measured by detecting the produced reducing sugar using DNSA (3,5- dinitrosalicylic acid) according to Miller [12]. One enzyme unit is defined as the amount of enzyme which liberates 1 μ mole of reducing sugar (glucose equivalent) per min at standard conditions. Protein had been measured according to Bradford [13].

2.3. PEGylation technique

The PEGylation procedure was done by mixing 0.5 ml of dextranase (2 mg/ml) in 20 mM Tris buffer (pH 8) with 0.5 ml of mPEG in an Eppendorff tube at room temperature for 10, 20, 30, 60 and 180 min. Two hundred microliter of the reaction mixture were added to 50 μl of 0.2 M Bis–Tris buffer (pH 5), to lower the pH to stop the reaction, and then 200 μl of this mixture was applied to Superose 12 HR10/30 column and the column was equilibrated with 20 mM Bis–Tris buffer (pH 5) containing 0.1 M NaCl. This experiment was done for mPEG 5000 and 30,000 Da with different concentrations (from 5.8 up to 116 mg/ml).

The pH adjustment of dextranase enzyme to 8 was done using 20 mM Tris buffer pH 8 by exchanging the buffer solution using concentration with Amicon Ultrafiltration cell and dilution using Tris buffer several times to a final product of 2 mg/ml dextranase in Tris buffer (pH 8). mPEG was dissolved in the same buffer.

2.4. Molecular weight estimation

Molecular weight determination was carried out using SDS/PAGE Phastgel gradient 8-25 electrophoresis using low molecular weight protein kits to detect the change in the molecular weight of dextranase after PEGylation.

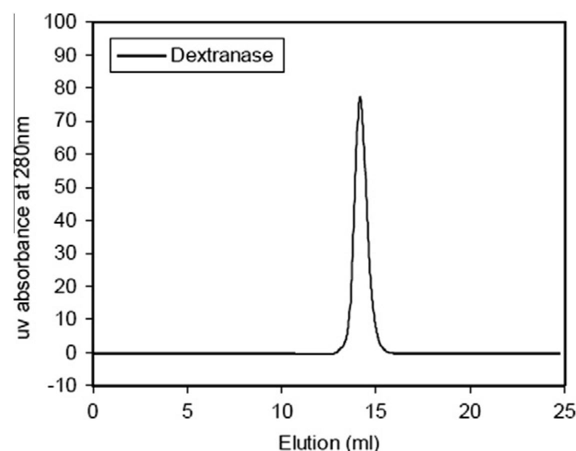

Figure 1 Size exclusion chromatography on Superose 12. A sample of 200 μL of a previously purified dextranase (0.45 mg/ml protein) was loaded to Superose 12 FPLC column (~23 ml). Linear flow rate 1 ml/min. UV absorbance at 280 nm.

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