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Short communication

Purification of α-L-arabinofuranosidase using a single-column mini-scale isoelectric focusing unit

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Abstract

Fabrication of a single-tube mini-scale isoelectric focusing unit with useful modifications is described. Provision of a side-arm to the unit facilitated easy collection of protein samples without disturbing the density and pH gradients. An ion-conducting polymer containing ether and hydroxy groups was fixed at the anodic end of the unit that separated the anodic solution from other solutions and also provided an easy path for charge transport. Using this unit, α-L-arabinofuranosidase (EC 3.2.1.55) was purified from crude broth of *Sclerotium rolfsii* in three steps with 33-fold purification.

1. Introduction

The overall operations and manoeuvring of the commercial instrumentation for electrofocusing of proteins prohibit their use for routine experimentation, hence Weller et al. [1] described a simple U-shaped mini-scale apparatus in which the p*I* of enzymes could be determined. Despite many advantages, the mini-scale units lack proper facilities for the collection of fractions from the column. This step must be followed meticulously without disturbing the pH and density gradient in order to achieve maximum resolution and recovery of the proteins. We have developed an improved single-tube electrofocusing column in which a special ion-conducting polymer is used. Using this column, α-L-arabinofuranosidase was purified to homogeneity in three simple steps.

2. Experimental

2.1. Strain and medium

Maintenance and cultivation of *Sclerotium rolfsii* were described previously [2]. The activity of α-L-arabinofuranosidase (α-L-AF) in international units (U) was measured using *p*-nitrophenyl α-L-arabinofuranoside (Sigma, N-3641) as the substrate.

2.2. Electrofocusing unit

The unit consists of single main column provided with a side-arm for easy collection of

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protein fractions without disturbing the pH and density gradient. This unit is provided with an ion-conducting polymer at the anodic end.

The following solutions were prepared: (A) electrolyte solutions, (i) 0.1 *M* phosphoric acid as anode solution and (ii) 0.1 *M* sodium hydroxide as cathode solution; (B) separation solution, 1.5 ml of glycerol + 1 ml of water; (C) gradient solutions, (i) high-density solution, consisting of 3 ml of glycerol, 2 ml of water, 0.2 ml of carrier electrolyte and the protein sample (10–100 μg) and (ii) low-density solution, consisting of 5 ml of water, carrier 0.2 ml of electrolyte and the protein sample (10–100 μg); and (D) the ion-conducting polymer, which was prepared as follows. A prepolymer was prepared by heating a mixture of medium molecular mass (2000–4000) ethylene glycol (4.0 g) 1,2,6-hexanetriol (0.4 g) and $FeCl_3$ (10 mg) for 4 h at 90°C and precipitating the mass in acetone to obtain a powder. This powder was mixed with poly(vinyl alcohol) (molecular mass 50 000) in the proportion of 30% (w/w) and blended thoroughly. A 2-g amount of the resulting blend was mixed with concentrated phosphoric acid (0.5 ml) and allowed to soak for 1 h to obtain a paste, which was applied to the end of an isoelectric focusing tube [3]. The various layers of the solutions are shown in Fig. 1.

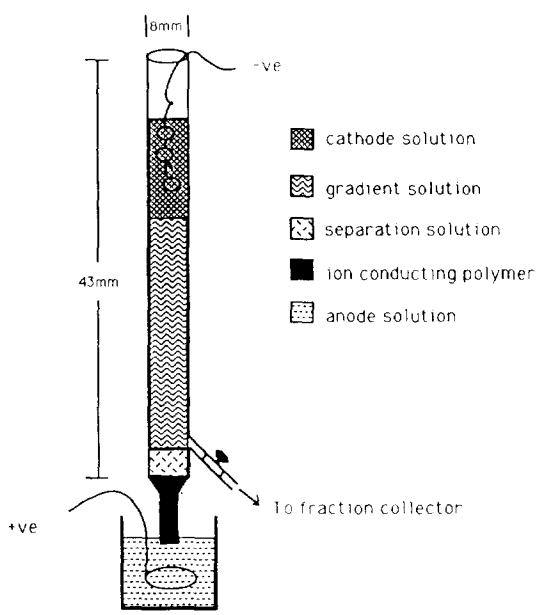

Fig. 1. Schematic diagram of mini-scale electrofocusing unit.

The apparatus assembly was placed in a cold room (5 ± 2°C) and the separation solution was layered above the conducting polymer up to the level of the side-arm (S). Gradient solutions (i) and (ii) were layered on the separation solution using a gradient mixer. Cathode solution was layered gently above the gradient using a 10-ml syringe. The platinum electrodes were dipped into respective solutions. Isoelectric focusing (IEF) was carried out for 8–10 h at 400 V, giving a current of 1.5 mA. Electrofocusing was discontinued and fractions of 0.2 ml each were collected by opening the tap on the side-arm. p*I* was determined by checking the pH of each fraction; protein can be detected either by measuring the absorbance at 280 nm or by carrying out an enzyme assay.

3. Results and discussion

3.1. Single-column IEF unit

It is possible to isolate and purify various protein samples conveniently using the improved apparatus shown in Fig. 1. The use of a side-arm with a tap is convenient for the collection of protein fractions. This design avoids many constraints during experimentation and its fabrication is simple.

3.2. Ion-conducting polymer

The anodic end of the IEF unit contains a layer of ion-conducting polymer, which has two functions: (a) it separates the anodic solution from the rest of the solutions and (b) it provides an easy path for charge transport. Ordinary gels or semipermeable membranes would normally create a large potential drop across them and decrease the net current flowing in the cell, giving rise to low efficiency. Hence a polymer containing ether and hydroxy groups was chosen, which would complex with the anodic solution (0.1 *M* phosphoric acid) and form an ionically conducting medium. Typically, a paste was made from 1 g of polymer powder and 0.7 ml of 1 *M*

phosphoric acid (85%) and fixed at the lower end of the column. It was ensured that the unit is leak-proof. The polymer was soaked in phosphoric acid for 1 h before the experimentation, which increased the conductivity owing to swelling and ionic dissociation. We have observed that the ion-conducting polymer shortens the duration of IEF: most models generally required 24–72 h but this has been shortened to 8–10 h.

3.3. Purification of α-L-AF

Crude broth of *S. rolfsii* was concentrated by ultrafiltration using an Amicon XM-300 membrane. The concentrate was subjected to Sephacryl S-300 gel column chromatography (104 × 1 I.D. cm). The fractions exhibiting high specific activity of α-L-AF were pooled and dialysed in a collodion bag (Sartorius SM 13200) for 8 h against 1% glycine solution. Finally dialysed enzyme was loaded for electrofocusing. The homogeneity of the enzyme was ascertained by observing a single protein band in polyacrylamide gel electrophoresis and also in analytical IEF in polyacrylamide gel. The steps of enzyme purification are summarized in Table 1.

α-L-AF is of increasing importance in biopulping, structural studies of carbohydrates [4] and cancer chemotherapy [5]. Therefore, screening for the microbial α-L-AF having the desired properties needs small amounts of a homogeneous or a highly refined preparation for preliminary characterization. The unit described here was useful for this purpose.

Commercial electrofocusing units need proportionately large amounts of expensive carrier electrolytes and a long period for the formation of a pH gradient, which often results in a loss of enzyme activity. To overcome these constraints, the modified single-column mini-scale IEF unit described here can be used routinely.

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Table 1
Purification of α-L-arabinofuranosidase from *S. rolfsii*

Step	Total activity (U/ml)	Total protein (mg/ml)	Specific activity	Purification (-fold)	Recovery in each step (%)
Crude broth	7.5	115	0.06	–	–
XM-300 concentrate	7.2	104	0.07	1.1	96
Sephacryl S-300	0.4	0.56	0.71	11.8	57
IEF	0.05	0.025	2.0	33.3	83