Asia Pacific Journal of Molecular Biology and Biotechnology, 2005
Vol. 13 (1) : 59-62

The comparative recovery performance of anion exchange and dye-ligand fluidised bed adsorption of G3PDH from unclarified yeast extract

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Received 21 March 2005 / Accepted 12 May 2005

Abstract. The comparative recovery performance of anion exchange and dye ligand fluidised bed adsorption of intracellular enzyme, glyceraldehydes 3-phosphate dehydrogenase (G3PDH) from unclarified disrupted yeast has been undertaken. The commercially available anion exchanger, Streamline QXL and the kiesleguhr-agarose composite adsorbent, Microsorb K6AX derived with dye-ligand (Cibaron Blue 3GA) were employed in fluidized bed experiments. The adsorbents were evaluated in respect of recovery performance in terms of yield, purity and enzyme specific activity.

Keywords. anion exchange, dye-ligand, Cibacron Blue 3GA, fluidised bed adsorption, G3PDH

INTRODUCTION

Ion exchange chromatography is one of the most common chromatographic techniques available for the primary separation and purification of charged proteins. The basis for ion exchange chromatography is the reversible association of charged groups located on protein molecules to oppositely charged functional groups immobilised on a stationary adsorbent phase and maintained over useful pH ranges (pH 5 – 9). The major disadvantage of such an approach was the lack of product selectivity of the anion exchanger, which shows affinity for all negatively charged components present in the feedstock. Cibacron Blue 3GA has been employed as a ligand for recovery of protein products including dehydrogenase, kinases (Stead, 1991) and albumin (Zhang and Sun, 2002). The dye interacts with proteins based on a rational mimicking of natural ligands and since they have no biological relations with the target protein, the term "pseudo-affinity" has been commonly adopted to describe them. The dye is a particularly promising pseudo-affinity ligands since it offers several advantages over biospecific ligand including ease of coupling to support matrices, low cost, wide availability and high stability (Prestera *et al.*, 1992).

Desorption of bound products in ion exchange adsorption is commonly achieved by unspecific elution using NaCl. The strength of adsorption between solute molecules for the charged groups on the adsorbent is decreased and resulted in eluting of bound proteins.Desorption of bound protein in dye adsorption can be achieved by either means of specific or unspecific elution. For example, the free nucleotide can be employed as a specific eluant to recover dehydrogenase adsorbed to immobilised dyes (Champluvier and Kula, 1992). The apparent competitive bioaffinity interactions of NAD^+ and dye contributed to the selective dissociation and elution of bound product (Boyer and Hsu, 1993). Unspecific eluants such as NaCl, KCl, and KSCN perturb molecular conformations and thus weaken the interaction between protein and dyes, contributing to protein desorption. The unspecific elution is usually performed better than specific elution and the unspecific eluants are relatively cheap.

In the present communication, a comparative study of dye-ligand and anion exchange recovery of intracellular proteins from unclarified yeast extract was made, wherein the Streamline QXL anion exchanger (ρ = 1.2 g ml^{-1}) and kiesleguhr-agarose

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Table 1. Fluidised bed anion exchange and dye-ligand recovery of G3PDH from unclarified yeast extract. The fluidized bed experiments were carried out as described in the text. The value in bracket represented the recovery performance achieved by dye-ligand adsorption.

	Biomass concentration (% ww/v original cells)		
	7.5	11.5	15
Ionic strength (mS cm^{-1}, 22.5°)	2.1	2.8	90.8
Original activity in feedstock (IU ml^{-1})	45.7	63.7	90.8
Original total protein concentration (mg ml^{-1})	2.3	4.3	7.5
Feedstock enzyme specific activity (IU mg^{-1})	19.9	14.8	12.1
Yield of G3PDH (%)	49.5	39.5	44.4(67)
Product enzyme specific activity (IU mg^{-1})	29.5	19.2	10.3(27.5)
Purification factor	1.5	1.3	0.9(2.3)

composite adsorbent (ρ = 1.3 g ml^{-1}) derived with Cibacron Blue 3GA (Zhang, 1999) were used in commercially available UpFront fluidised contactor (20 mm i.d.). The purification of an intracellular enzyme, glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was selected as a suitable target for comparative testing of adsorption systems.

Fluidised bed adsorption of G3PDH was performed using the UpFront contactor at room temperature. The feedstocks comprising 7.5 % - 15 % equivalent wet cell weights (pre-wet milling) at pH 7.5 were studied herein. Streamline QXL and Macrosorb K6AX Cibacron Blue 3GA (50 ml settled volume, corresponding to 15.5 cm settled bed height) were loaded into the contactor and equilibrated with 10 mM Tris/HCl, pH 7.5 containing 1 mM EDTA (buffer A) in fluidised bed mode. Equilibration of the adsorbent bed was judged to be complete when the pH and conductivity of the column effluent was equivalent with the starter buffer A. After the equilibration step, the feedstock was loaded to the bed at the linear velocity (50 to 200 cm h^{-1}). The washing stage was performed after the feedstock application operation. Then the adsorbent was allowed to settle, and desorption of bound G3PDH was carried out in packed bed mode. Elution was carried out in single-step method exploiting 0.15 to 0.2 M NaCl in buffer A. Representative fractions (1 ml) were collected and assayed for enzyme activity and total protein concentration as described in a previous publication (see Jahanshahi *et al.*, 2002).

Table 1 summarises the comparative elution performance of the purification process. Higher biomass concentration (i.e. from 7.5% to 15% ww/v) and ionic strength apparently created more competition for enzyme binding from feedstock to anion exchanger. The consequence of lack of selectivity is that many negatively charged species e.g. cell debris, cells, pigments and proteins present in the unclarified yeast homogenate is bound to Streamline QXL. The dark brown fouling of Streamline QXL (originally white in colour) confirmed such an observation. As a result the adsorption performance was diminished (i.e. from 7.5% to 15% ww/v, see Table 1). The elution yield obtained at 15 % ww/v biomass was 44 % and 67 % of bound material after washing for anion exchange and dye-ligand adsorption respectively. The difference in yields can be considered significant, with a margin of 23 %. The dye-ligand adsorption also yielded a higher purification factor of 2.3. The better recovery performance achieved by dye-ligand adsorption suggested that the interference of cell debris and other negatively charged macromolecules were reduced considerably when using a more selective adsorption system (see Table 1). Hence, the available ligand capacity for G3PDH adsorption was not greatly reduced by impurities and contaminants in the adsorption process involving 15 % ww/v yeast disruptate. This was confirmed by visual inspection since the adsorbent particles remained close to the original blue colour at the end of the washing stage. The early work of Chase and Draeger (1992) exploring the presence of intact yeast cells on BSA adsorption revealed that the cells had more adverse effects on ion exchange systems than on the specific affinity systems. In addition, the better adsorption performance seen here (i.e. 15 % ww/v biomass) might be attributed to the dye-ligand system, that is less sensitive to the high ionic strength of the feedstock. It has been reported by a several researchers (Thoemmes *et al.*, 1996; Hamilton *et al.*, 2000) that the high ionic strength of the feedstock could greatly diminish the adsorption based on ion exchange system.

The success of the primary recovery of G3PDH from unclarified 15 % ww/v yeast extract was documented by analysing the dissociated polypeptides contained in selected samples obtained during the purification process employing SDS-PAGE analyses (Figure 1). G3PDH would be expected to be represented by the detergent dissociated 36,000 subunit of the tetrameric enzyme with native molecular mass of 146,000 daltons (Harrington and Corr, 1965). The superior purity of enzyme obtained by pseudo-affinity purification is clear when comparing lanes 3 and 5 in Figure 1. There are more proteins co-eluted in anion exchange purification system.

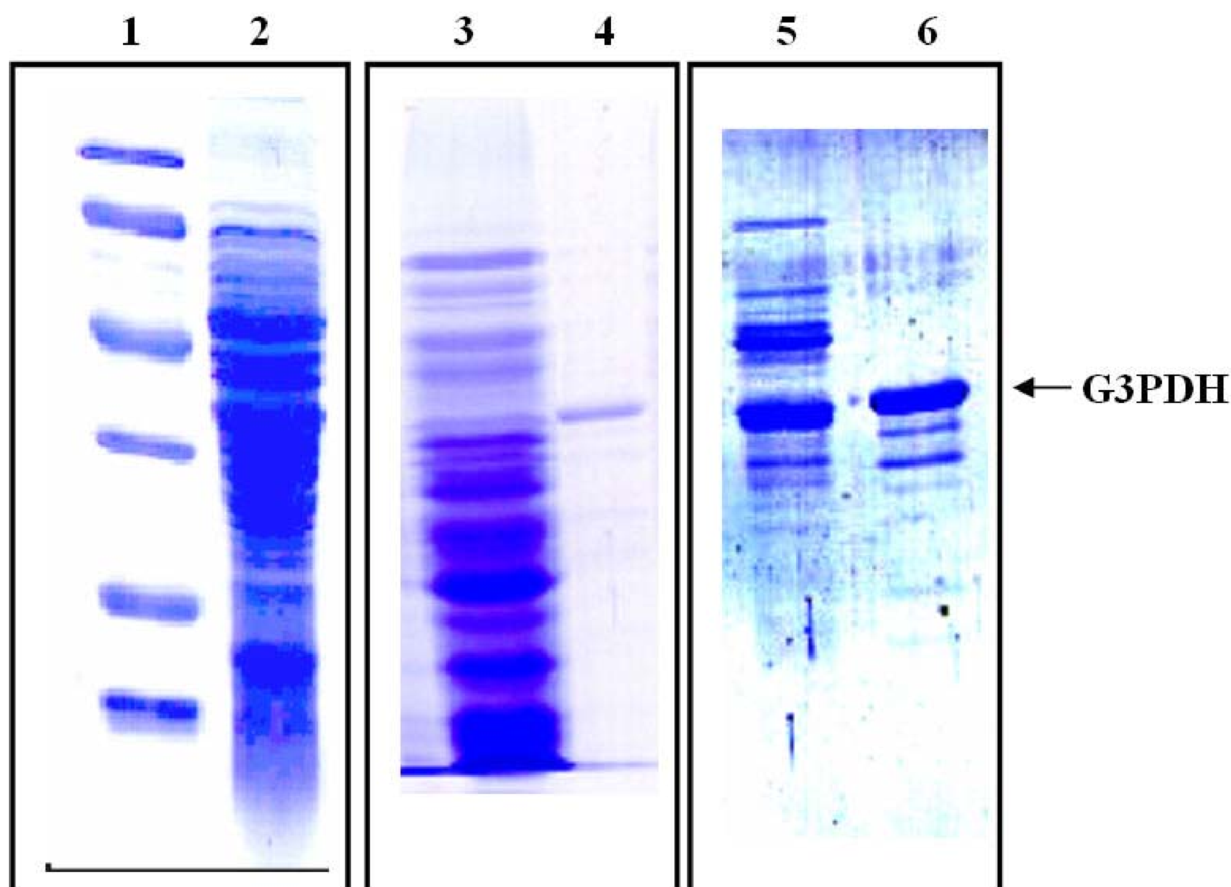

Figure 1. SDS-PAGE analysis of recovered G3PDH from unclarified yeast extract (15 % ww/v). The samples were analysed by SDS-PAGE. Lane 1 contains the standard low molecular weight (LMW) marker from Pharmacia (Uppsala, Sweden). Lane 2 carries the initial yeast disruptate (15 % frozen wet weight/volume original cells). Lane 3 carries the eluted product (0.15 M NaCl) from Streamline QXL and lane 5 carries the eluted product (0.2 M NaCl) from Macrosorb K6AX Cibacron Blue 3GA. Lanes 4 and 6 depict the commercial rabbit muscle G3PDH from Sigma (Pool, UK).

The presence of a strongly staining band with an electrophoretic mobility approximating to that of rabbit muscle G3PDH (no yeast standard for this enzyme is commercially available) indicated the concentration and primary purification of G3PDH from unclarified feedstock was achieved. However, the presence of a strong band with an electrophoretic mobility approximating to 43,000 daltons and other minor bands might indicate lack of complete selectivity of Cibacron Blue 3GA for G3PDH. Cibacron Blue 3GA has been demonstrated to interact with several enzymes potentially present in yeast such as aldehyde dehydrogenase, 228 KD (Tamaki *et al.*, 1977), glucose-6-phosphate dehydrogenase, 128 KD (Champluvier and Kula, 1992) and alcohol dehydrogenase, 150 KD (Mohammed *et al.*, 1995).

It has been clearly demonstrated that dye-ligand adsorption is a feasible method for the primary recovery of intracellular enzymes such as G3PDH from unclarified yeast extract. Use of this pseudo-affinity method has enabled the enzyme to be more selectively and efficiently captured from the crude feedstock, indicating that there was less interference of negatively charged components present in the feedstock with this adsorbent. Additionally, the better adsorption performance achieved here might be attributed to the fact that dye-ligand was less sensitive to the ionic strength of feedstocks.

Acknowledgements

Tau Chuan Ling gratefully acknowledges financial support from Universiti Putra Malaysia, Malaysia and the School of Chemical Engineering, University of Birmingham, UK.

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