AFFINITY CHROMATOGRAPHIC ISOLATION OF THE PERIPLASMIC MALTOSE BINDING PROTEIN OF *ESCHERICHIA COLI*

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1. Introduction

The periplasmic, osmotic-shock releasable maltose binding protein is one of at least four gene products that make up the maltose transport system of *Escherichia coli* [1]. A further component is an outer membrane protein known as the phage λ receptor [2] which is obligatory for the permeation of longer maltodextrins into the cell [3]. The gene products of the *mal*F and *mal*K genes are also necessary for maltose transport [4] and at least the *mal*F product is likely to be an inner membrane protein [5]. In addition to its transport function, the periplasmic binding protein also acts as chemoreceptor in taxis towards maltose [6].

In order to study the proteins involved in the process of transport (and chemotaxis), it was of interest to prepare an affinity chromatographic material satisfying the substrate requirements of the transport system (and chemoreceptor). It has been demonstrated that the maltose transport system is also responsible for the entry of maltodextrins into the cell and that the binding protein recognizes not only maltose but also maltodextrins up to at least maltoheptaose [3]. This communication demonstrates that amylose crosslinked by epichlorohydrin also satisfies the substrate specificity of the maltose binding protein and hence provides a simple affinity chromatographic substrate for studies of maltose recognition in transport and chemotaxis. It also permits a rapid, one step purification of the maltose binding protein from osmotic-shock fluids.

2. Methods

2.1. *Preparation of crosslinked amylose*

The crosslinking of amylose was adapted from a method described for starch [7]. Amylose, 1 g (Sigma, grade III, from potato) was evenly suspended in 4 ml water and warmed to 50°C in a water bath. NaOH, 6 ml 5 N, followed by 3 ml epichlorohydrin were added with rapid stirring. The suspension solidified into a gel within about 10 min, was allowed to cool to room temperature and left for 45 min. The gel was washed with water, transferred to a Waring blendor and fragmented by brief blending as a suspension in water. The broken gel was transferred to a measuring cylinder and suspended in 3 separate 100 ml washes of buffer containing 50 mM glycine–HCl, 0.5 M NaCl, (pH 2.0). Between each wash, the gel was allowed to settle to separate fines remaining in the supernatant. Two further fining cycles in water and two in 10 mM Tris–HCl buffer (pH 7.2) were performed. Finally, the gel was stored by suspension in 10 mM Tris–HCl (pH 7.2) containing 0.02% (w/v) sodium azide.

2.2. *Purification of maltose binding protein*

Maltose binding protein was isolated from the wild-type *Escherichia coli* K12 strain Hfr G6 [1]. Bacteria were grown into late exponential phase on minimal salts medium A containing 0.2% maltose as sole carbon source [8]. Osmotic-shock fluids were prepared as in [9]. The dilute shock fluid was filtered through a 0.45 μm membrane filter to remove whole

bacteria and concentrated by lyophilisation. The concentrated shock fluid was extensively dialyzed against 10 mM Tris–HCl buffer (pH 7.2) before affinity chromatography (fig.1). The protein peak eluted by maltose was combined and lyophilised. The redissolved protein in 10 mM Tris–HCl buffer (pH 7.2) was extensively dialyzed against the same buffer and stored frozen at −15°C. Maltose binding protein purified by the method in [1] was kindly provided by W. Boos.

2.3. *Maltose binding assays*

The detection of binding activity by equilibrium dialysis towards [^{14}C]maltose was as in [1]. The substrate-dependent fluorescence quenching exhibited by maltose binding protein [3] was used to estimate the dissociation constant of maltose binding. Measurements were performed using a Perkin-Elmer MPF 3 fluorescence spectrophotometer at 21°C with excitation at 280 nm and emission followed at 350 nm. Both excitation and emission slits were set at 8 nm. The conditions of assay were as in [3].

2.4. *Gel electrophoresis*

Sodium dodecyl sulphate (SDS)–acrylamide gel electrophoresis [10], acrylamide gel electrophoresis in 8 M urea in the buffer systems at pH 8.5 [11] and at pH 2.3 [12] were by the published methods. All gels contained 7.5% acrylamide. Protein bands were stained with Coomassie blue R 250 and gels destained as in [10]. Bands were detected by scanning stained gels in a Gilford 250 spectrophotometer at 550 nm.

2.5. *Antibody preparation*

Antiserum to pure binding protein was prepared by injecting a rabbit with two doses each containing 1 mg protein in 0.5 ml 10 mM Tris–HCl buffer, pH 7.2, mixed with an equal volume of Freunds complete adjuvant. The injections were 3 weeks apart and serum was isolated 4 weeks after the second injection.

Protein estimations were by the Lowry method [13].

3. Results

Preliminary equilibrium dialysis experiments demonstrated that inclusion of amylose in a dialysis bag with concentrated shock fluid from maltose-grown bacteria resulted in a strong inhibition of [^{14}C]maltose binding. This suggested that amylose could at least compete for maltose binding sites in the shock fluid. To test whether epichlorohydrin crosslinked amylose could also interact with the maltose binding protein, concentrated shock fluid was subjected to chromatography as shown in fig.1. It was found that no maltose binding activity was eluted with starting buffer in the major peak of protein unabsorbed by amylose. Elution with 10 mM maltose resulted in the appearance of a peak containing about 20% of the applied protein. The second peak could also be eluted by 10 mM maltotriose but not 10 mM glucose, consistent with the known binding properties of the maltose binding protein [1].

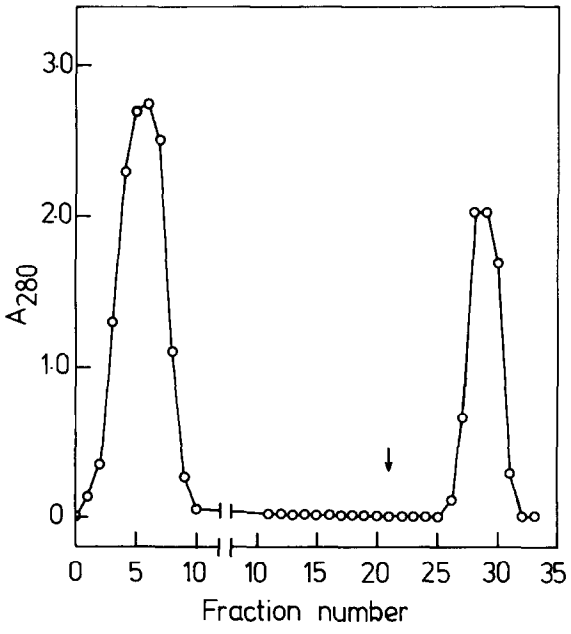

Fig.1. Chromatographic separation of maltose binding protein. A column, 9.5 × 2.6 cm, of crosslinked amylose was equilibrated with 10 mM Tris–HCl (pH 7.2). Concentrated shock fluid protein (60 mg) dialyzed against the same buffer was applied. Elution with 10 mM Tris–HCl (pH 7.2) continued until no further protein was eluted from the column and at the point indicated, the elution buffer was changed to 10 mM Tris–HCl containing 10 mM maltose (pH 7.2). The fraction volumes were 10 ml up to fraction 10 and 2 ml thereafter.

Fig.2. Immunodiffusion test for the maltose binding protein. The centre well contained antibodies raised against protein retained by crosslinked amylose. The top outer well contained 24 μg maltose binding protein purified by the method in [1] and the two outer wells on either side each contained 25 μg of amylose-retained protein.

Three additional lines of evidence suggest that the protein retained by amylose was indeed the maltose binding protein isolated by conventional methods.

1. Antibodies raised against the newly isolated protein were tested for cross-reactivity against protein isolated by the method in [1]. In double diffusion experiments, the antibodies reacted identically towards protein prepared by either method (fig.2).

2. The fluorescence emission spectra of the newly isolated protein were identical to those in [3]. The characteristic quenching patterns and peakshifts with maltose and maltodextrin were also observed (fig.3).

3. The dissociation constant for maltose, estimated from substrate-dependent fluorescence quenching, was 1.9 μM, consistent with values obtained from fluorescence [3] and equilibrium dialysis experiments [14].

The purity of the protein eluted with maltose was tested by acrylamide gel electrophoresis as shown in fig.4. A single band of protein was observed in gels run in the presence of 8 M urea, at pH 8.5 and pH 2.3, and in 0.1% SDS. From separate 7.5% SDS–acrylamide gels calibrated with muscle aldolase (40 000 mol. wt), ovalbumin (43 000 mol. wt), serum albumin (68 000 mol. wt) and β-galactosidase (130 000 mol. wt) a molecular weight of 40 000 was obtained for the protein, consistent with the value in [1].

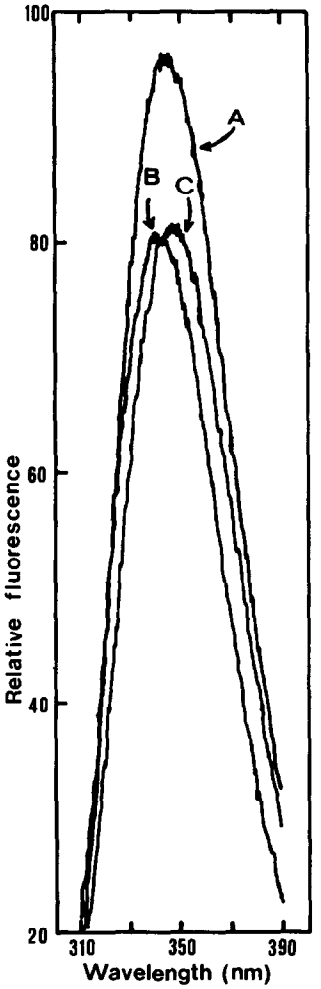

Fig.3. Fluorescence emission spectra of the amylose binding protein, and the quenching effect of maltose and maltodextrin. Line A: relative fluorescence of a 20 μg/ml solution of binding protein in 10 mM Tris–HCl, 2 mM $MgCl_2$ buffer (pH 7.2). Line B: fluorescence in the presence of added mM maltoheptaose. Line C: further addition of 1 mM maltose.

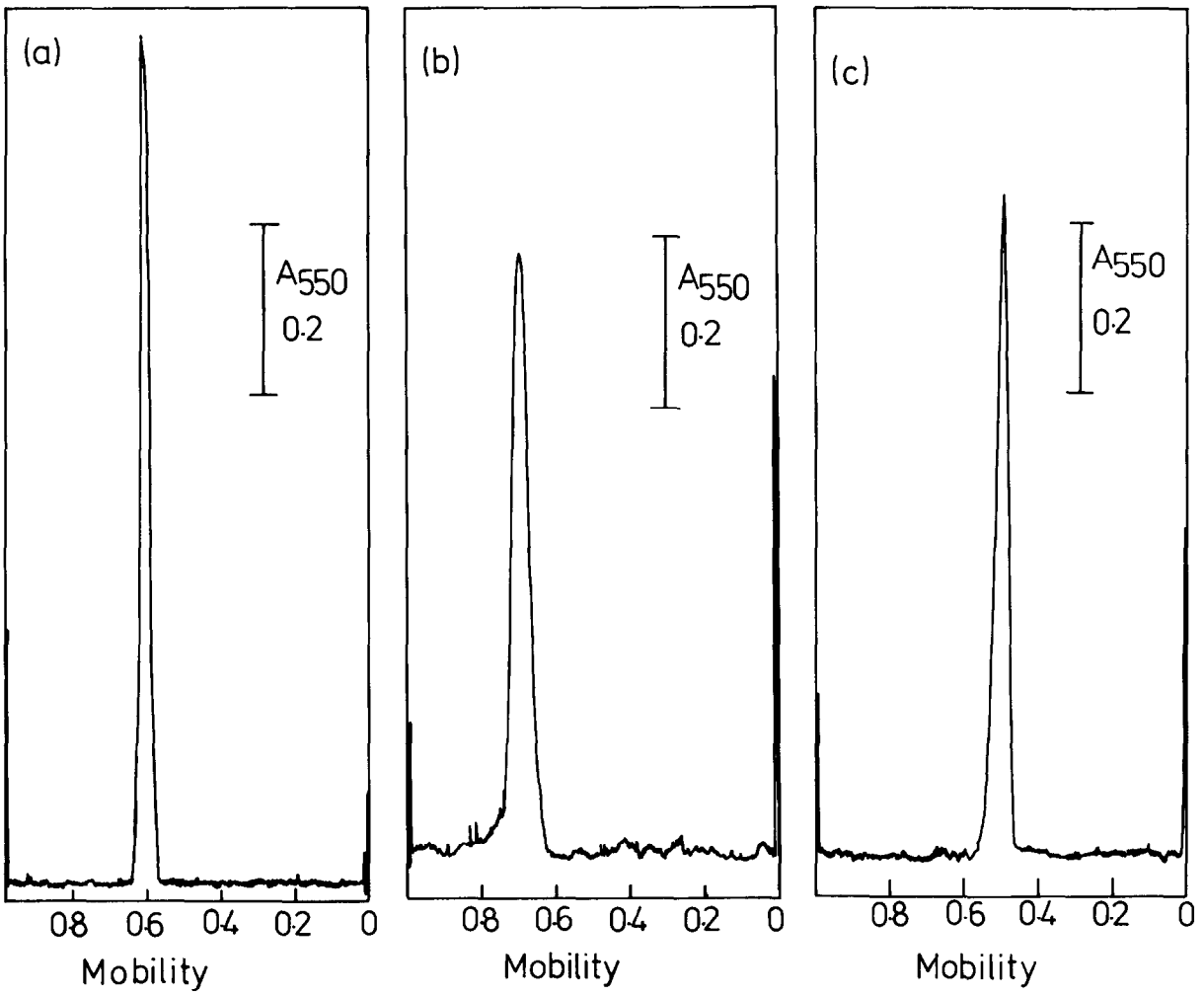

Fig.4. Scans of the purified maltose binding protein preparation after gel electrophoresis. All gels were loaded with 20 μg total protein. Electrophoresis in the presence of: (a) 0.1% SDS, at pH 7.2; (b) 8 M urea, at pH 2.3; (c) 8 M urea, at pH 8.5.

4. Discussion

Using crosslinked amylose, a simple purification of the periplasmic maltose binding protein has been developed. The protein isolated by this method was electrophoretically homogeneous and recovered in high yield. From fully induced cells, about 20% of the protein in shock fluids was recovered as maltose binding protein; more than 12 mg pure protein could be isolated from a 10 l late exponential culture containing about 12.5 g wet wt bacteria.

Affinity chromatographic approaches have not been as successful in isolating bacterial transport proteins. In the case of periplasmic binding proteins, an affinity chromatographic step has been used in the purification of the phosphate binding protein of *E. coli* [15], but additional steps were necessary to get pure protein. The *his*T protein of *Salmonella typhimurium* has also been partially purified using hydrophobic chromatography [16].

In future, given that crosslinked amylose fulfils the substrate-binding requirements of the maltose

transport system, it should be possible to test whether other membrane-bound components of the system also interact with amylose. Such an interaction would offer a simple method of purifying these unidentified components. In addition, the ready availability of large quantities of pure maltose binding protein should facilitate studies of its properties and possible interaction with membrane components involved in transport and chemotaxis.

Acknowledgement

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