PREPARATION OF THE DNA-BINDING PROTEIN HU FROM *ESCHERICHIA COLI* BY IMMUNO-AFFINITY CHROMATOGRAPHY

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1. Introduction

The protein HU is a low molecular weight DNA-binding protein which has been characterized in *Escherichia coli* cells [1–3]. The isolation of this protein requires usually three steps of chromatography [1,2,4], which are time-consuming and led to a low recovery of pure protein.

We describe here a very selective method based on immuno-affinity chromatography to obtain in a single step highly purified protein HU. This method uses a column of purified anti-protein HU antibodies coupled to Sepharose 4B.

2. Materials and methods

All chromatographic separations were performed at room temperature.

2.1. *Preparation of the anti-protein HU column*

Monospecific antiserum against protein HU was prepared by immunizing a rabbit with 0.5 mg protein HU isolated as in [4]. Each injection contained 0.1 mg antigen dissolved in 0.1 ml 0.15 M NaCl and emulsified with 0.1 ml Freund's complete adjuvant (Difco).

Rabbit anti-protein HU antiserum was repeatedly applied in 10 ml fractions on a protein HU–Sepharose column obtained by coupling 11 mg protein HU with 30 ml Sepharose 4B pre-activated according to the CNBr method in [5]. The column was washed with 0.01 M sodium phosphate buffer (pH 7.2) containing 0.15 M NaCl and 0.02% NaN_3 (buffer 1) until the $A_{280\ nm}$ returned to the baseline. The bound anti-protein HU antibodies were then desorbed with 0.2 M glycine–HCl (pH 2.8) containing 0.5 M NaCl (buffer 2). The solution of antibodies was neutralized with 1 M NaOH, dialyzed against deionised water and freeze dried.

In the next step, the anti-protein HU antibodies (60 mg) were coupled with 50 ml CNBr-activated Sepharose 4B.

2.2. *Protein HU purification*

Method 1. *E. coli* cells (strain W 3350, Institut Pasteur collection) were suspended at + 4°C in 20 mM Tris–HCl buffer (pH 8.0) containing 1 mM Na_2 EDTA and 1.7 M NaCl (buffer 3) and broken in a French press with a pressure of 17 000 p.s.i. The high salt concentration is necessary to dissociate the nucleoprotein complexes. The crude extract was centrifuged at 8000 × *g* to remove the cell debris and then clarified at 50 000 × *g* for 3 h. The supernatant was applied directly on the anti-protein HU antibodies column. The column was washed with buffer 3. The bound protein HU was then desorbed with buffer 2. The protein solution was desalted by gel filtration chromatography on a column (100 × 2.5 cm) of Sephadex G-25 fine equilibrated and eluted with 0.01 N HCl.

Method 2. The *E. coli* cells were suspended in 20 mM Tris–HCl buffer (pH 8.0) containing 1 mM Na_2 EDTA, 0.01 M $MgCl_2$, 2 mM $CaCl_2$ and broken in a French press. The extract was then incubated at 10°C for 90 min in presence of pancreatic

Abbreviations: CNBr, cyanogen bromide; EDTA, ethylene diamine tetracetate, SDS, sodium dodecyl sulfate

deoxyribonuclease I (Worthington) (20 μg/ml). The extract was clarified by centrifugation and was made 0.4 M in NaCl prior to application on the anti-protein HU antibodies column. The column was washed with 20 mM Tris–HCl buffer (pH 8.0) containing 1 mM Na_2 EDTA and 0.4 M NaCl. The bound protein was then further treated as in method 1.

Method 3. The material eluted with 0.4 M NaCl from a DNA-cellulose column [1] was applied on the anti-protein HU antibodies column. In this procedure, the column was washed with 20 mM Tris–HCl buffer (pH 8.0) containing 1 mM Na_2 EDTA and 0.4 M NaCl and the bound protein recovered as in method 1.

2.3. *Polyacrylamide gel electrophoresis*

The purity of the protein was assessed by SDS–polyacrylamide slab gel electrophoresis [6] using the Laemmli buffer system [7] and by electrophoresis on cylindrical polyacrylamide gels (0.5 × 12 cm) performed in 2.5 M urea, at pH 2.7 [8] using a 17% acrylamide concentration.

2.4. *Electroimmunodiffusion*

The elution of the protein HU from the column was monitored quantitatively by rocket immunoelectrophoresis [9] on anti-protein HU antibodies-impregnated agarose plates. Samples of 4 μl were applied at different dilutions. A linear standard curve from 10–50 μg/ml was used.

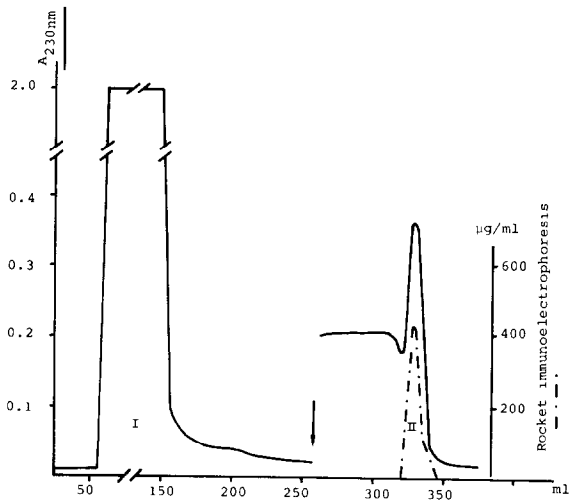

Fig.1. Affinity chromatography of a crude extract from *E. coli* cells on anti-protein HU antibodies bound to a Sepharose 4B column. An extract of *E. coli* cells was applied on the column. The column was washed with Tris–EDTA buffer pH 8.0 (buffer 3) until the A_{230} measured against buffer 3 returned to the baseline. The column was then eluted with glycine buffer pH 2.8 (buffer 2) and, from that moment, indicated by the arrow, the A_{230} of the eluate was measured against buffer 2 since the elution of protein HU cannot be detected by a measure at A_{230} against buffer 3. Therefore a temporary baseline shift of about 0.2 A unit due to EDTA in buffer 3 was observed before the elution of the protein HU (peak II) up to the complete disappearance of the buffer 3 in the eluate. The elution of protein HU was also monitored by rocket-immunoelectrophoresis of aliquot taken from each fraction on agarose gel impregnated with anti-protein HU antibodies (·–·). Flow rate was 40 ml/h and 5.0 ml fractions were collected.

3. Results and discussion

The elution pattern of the isolation of protein HU by affinity chromatography on anti-protein HU antibodies column is presented in fig.1. A large amount of material which has no affinity for the anti-protein HU antibodies is not retained by the column and is eluted in fraction 1. Fraction 2 corresponds to pure protein HU as assessed by polyacrylamide gel electrophoresis in presence of SDS (fig.2) and in presence of urea (fig.3).

In our experimental conditions, ~4.0 mg protein HU eluted in fraction 2 as estimated by rockets immunoelectrophoresis. The anti-protein HU antibodies column has been used over 6 months without noticable decrease of its capacity.

The purity of the protein HU obtained by affinity chromatography was also checked by amino acid analysis. The amino acid composition is identical to that given in [4].

Thus, the affinity chromatography on anti-protein HU antibodies column appears to be a valuable tool for the isolation of the protein HU. The use of monospecific antibodies for the preparation of the column and the use of high ionic strength conditions for the chromatography permits the elimination of non-specific interactions of protein contaminants. The high selectivity of the procedure permits the isolation

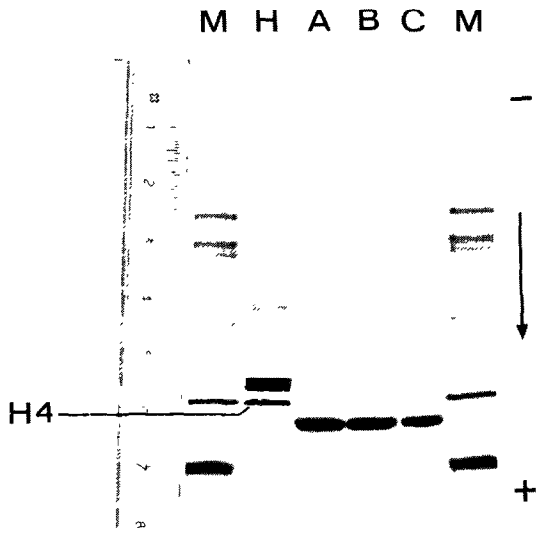

Fig.2. SDS electrophoresis of protein HU on a 5–30% polyacrylamide gradient gel: H, calf thymus whole histone; A, protein HU obtained from a crude extract (1.7 M in NaCl) of *E. coli* cells; B, protein HU obtained from a crude extract of *E. coli* cells, incubated with pancreatic DNase I; C, protein HU obtained from the material eluted with 0.4 M NaCl from a DNA-cellulose column. M, molecular weight markers: bovine serum albumin (68 000); IgG heavy chain (50 500); ovalbumin (43 000); IgG light chain (23 500); cytochrome *c* (12 500); insulin (5700). Samples (5 μg) were treated with 5% SDS, 2% 2-mercaptoethanol for 2 min at 100°C and run at 40 mA for 2 h. Electrode buffer (Tris/glycine, pH 8.3) and gel buffer (Tris/HCl, pH 8.9) were made 0.1% in SDS. The gel was stained with 0.25% Coomassie Blue R 250 in acetic acid–ethanol–water (10:45:45, v/v/v) and destained in acetic acid–ethanol–water (10:25:65, v/v/v).

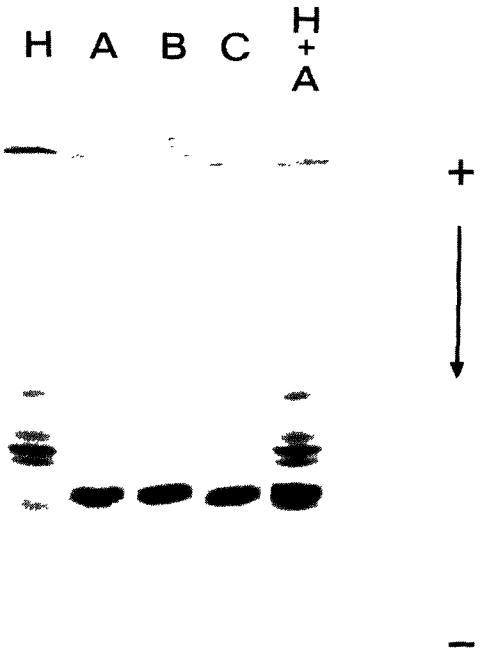

Fig.3. Polyacrylamide gel electrophoresis of protein HU. For H, A, B and C, see legend of fig.2. Electrophoresis was carried out at 1.5 mA/tube for 4 h, at pH 2.7, in 2.5 M urea [8] with the use of gels (0.5 × 12 cm) containing 17% acrylamide. Gels were stained for 30 min with 1% Amido Black 10B in acetic acid–ethanol–water (1:2:7, v/v/v) and destained for 48 h by diffusion in the same mixture.

of pure protein HU directly from a crude extract of *E. coli* cells (methods 1 and 2). Since several chromatographic steps which require dialysis and ultrafiltration are avoided, the procedure is much more rapid and gives better yields, when compared to those in [1,2,4]. However, although the methods 1 and 2 give very satisfactory results it might be advisable to use method 3 where the material applied to the anti-protein HU antibodies column has been partially purified by chromatography on DNA-cellulose in order to prolong the life of the column.

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