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Note

Chromatography of chromatin proteins on Cibacron Blue F3G-A–agarose

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Cibacron Blue F3G-A linked to agarose, either directly or as the dextran derivative Blue Dextran, has been widely used as an affinity chromatography matrix for the purification of a variety of nucleotide-requiring enzymes[1,2]. It has been proposed that the Blue Dextran–agarose matrix is specific for proteins containing the so-called "dinucleotide fold", while the Cibacron Blue–agarose matrix is less specific and may have affinity for all proteins with a nucleotide-binding site[3,4].

In this paper we show that most chromatin proteins from Ehrlich ascites tumour cells, both histones and non-histones, interact strongly with Cibacron Blue–agarose columns, and can be fractionated by elution with buffers containing variable amounts of sodium chloride and/or urea.

EXPERIMENTAL

Blue Sepharose CL-6B, a Cibacron Blue F3G-A–agarose matrix and Pharmacia LMW Electrophoresis Calibration Kit (a mixture of purified proteins with known molecular weights) were obtained from Pharmacia (Uppsala, Sweden).

Preparation of nuclei, chromatin and protein fractions

Ehrlich ascites tumour cells were propagated, nuclei were prepared and poly(ADP–ribose) polymerase was extracted and partially purified by chromatography on DNA–agarose as previously described[5]. The supernatant after the first treatment of the cells with Triton N-101, containing mainly proteins of cytoplasmic origin, was dialysed against 50 m*M* Tris–HCl buffer (pH 8.0) containing 1 m*M* dithiothreitol and 0.14 *M* sodium chloride, and precipitated material was removed by centrifugation. The proteins in the supernatant were assumed to be representative of cytoplasmic proteins generally.

Chromatin was prepared from the nuclei by the method described by Rickwood and Birnie[6]. Non-histone chromatin proteins were obtained by dissociating the chromatin in 20 m*M* Tris–HCl buffer (pH 7.7) containing 1 m*M* dithiothreitol and 1 *M* sodium chloride, followed by dialysis against 6 volumes of the same buffer without sodium chloride, and centrifugation[7]. The preparations were used directly after preparation, or after storage for a few days at −20°.

Histones were extracted from the nuclei with 0.25 *M* hydrochloric acid, after extraction of poly(ADP–ribose) polymerase with 175 m*M* potassium phosphate

(pH 8.5). They were precipitated by the addition of 8 volumes of acetone, dried and stored at room temperature. Prior to chromatography on Blue Sepharose, they were dissolved in water containing 1 m*M* phenylmethylsulphonyl fluoride to inhibit proteases. The solution was then adjusted to give a final concentration of 50 m*M* Tris–acid (pH 8.0), 1 m*M* dithiothreitol and 0.14 *M* sodium chloride.

Chromatography on Blue Sepharose

Poly(ADP–ribose) polymerase was purified by chromatography on Blue Sepharose as described elsewhere[8], by applying partially purified polymerase in 10 m*M* Tris–HCl buffer (pH 8.0) containing 10% glycerol, 1 m*M* dithiothreitol, 0.02% sodium azide and 0.25 *M* sodium chloride to a 10-ml column of Blue Sepharose equilibrated with the same buffer, washing with the same solution and elution of the polymerase with buffer containing 0.4 *M* sodium chloride. Proteins still retained were eluted with buffer containing 1 *M* sodium chloride, 2 *M* sodium chloride and 6.75 *M* urea–1 *M* sodium chloride.

Chromatography of other protein fractions was performed on a column of Blue Sepharose CL-6B (4.3 × 0.9 cm) equilibrated with 50 m*M* Tris–HCl buffer (pH 8.0) containing 1 m*M* dithiothreitol and 0.14 *M* sodium chloride. The chromatography was performed at room temperature with a flow-rate of about 25 ml/h.

Protein determination

Protein content was determined by the Lowry method, except for histones after precipitation of the protein with trichloroacetic acid in the presence of sodium deoxycholate[9]. Bovine serum albumin was used as a reference protein.

Polyacrylamide gel electrophoresis

Preparations of poly(ADP–ribose) polymerase were analysed in 5% polyacrylamide gels containing 0.9 *M* acetic acid and 4.5 *M* urea, as described previously[10].

Histones were analysed in the acetic acid–urea system of Panyim and Chalkley[11], with a urea concentration of 2.5 *M*. Prior to electrophoresis the histones were precipitated with 8 volumes of acetone (samples with high salt concentrations were diluted with water prior to the addition of acetone), and dissolved in 0.9 *M* acetic acid containing 2% 2-mercaptoethanol and 0.2 *M* sucrose. After electrophoresis the gels were stained with 0.1% amido black in 0.7% acetic acid, 30% methanol, and destained electrophoretically. The gels were scanned at 600 nm with a Beckman DU Model 2400 spectrophotometer equipped with a Gilford gel scanner and a W + W 600 recorder. The purities of the fractions were calculated from the areas under the peaks in the scans.

Non-histone chromatin proteins were analysed by electrophoresis in 8.75% polyacrylamide gel slabs, 1.5 mm thick, containing sodium dodecylsulphate[12].

RESULTS AND DISCUSSION

The results obtained by chromatography of cytoplasmic proteins and chromatin proteins on Blue Sepharose are given in Table I. As might be expected, most proteins of cytoplasmic origin were not retained under the conditions used. In contrast, chromatin proteins were retained to a high degree. Of the non-histones, 86%

TABLE I

FRACTIONATION OF VARIOUS PROTEIN PREPARATIONS ON BLUE SEPHAROSE

About 2 mg of protein were applied to the column in each experiment. After loading, the column was washed with 15 ml of buffer containing 0.14 *M* NaCl. The concentration of NaCl was then increased as indicated; in each instance 15 ml of eluate was collected before the salt concentration was increased. Prior to elution with urea-containing buffer, salt was removed from the column with 10 ml of salt-free buffer; no protein material was eluted during this wash. Bound cytoplasmic proteins were eluted directly with buffer containing urea and NaCl, without attempts at further fractionation. The results given are protein eluted as a percentage of material applied.

Protein preparation	*Molarity of NaCl in elution buffer [50 mM Tris–HCl (pH 8.0), 1 mM dithiothreitol]*					
	0.14 (unbound material)	*0.5*	*1.0*	*2.0*	*0.0 +6.75 M urea*	*1.0 + 6.75 M urea*
Cytoplasmic proteins	84					16
Histones	0	0	22	38	0	40
Non-histone chromatin proteins	14	27	20	9	7	8

of the applied protein was bound to the column in 0.14 *M* sodium chloride, while the histones were quantitatively retained. Thus, most chromatin proteins appear to have structural features which make them interact with Cibacron Blue under these conditions. The strength of the interaction between protein and ligand apparently

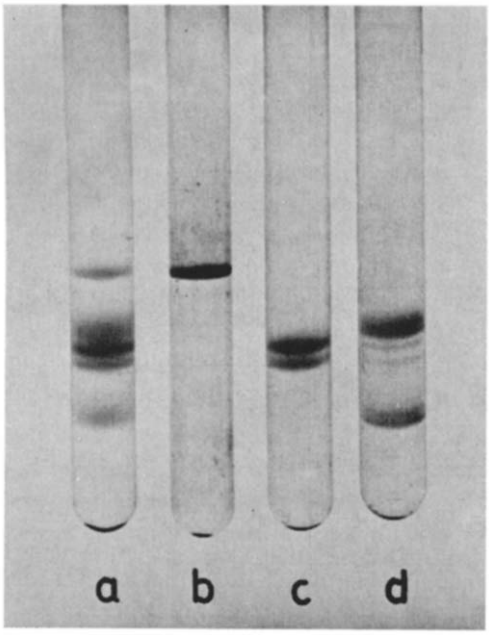

Fig. 1. Polyacrylamide gel electrophoresis of fractions obtained by chromatography of histones on Blue Sepharose. Eluent: 50 m*M* Tris–HCl (pH 8.0) containing 1 m*M* dithiothreitol and NaCl/urea as specified. (a) Unfractionated histones; (b) material retained with 0.5 *M* NaCl, eluted with 1*M* NaCl; (c) material retained with 1 *M* NaCl, eluted with 2 *M* NaCl; (d) material retained with 2 *M* NaCl and at 6.75 *M* urea, eluted with buffer containing 1 *M* NaCl and 6.75 *M* urea. Electrophoresis conditions: see Experimental.

varies from protein to protein, as some proteins can be eluted from the column with 0.5 *M* sodium chloride, while others demand high concentrations of both sodium chloride and urea for elution to occur.

The histone fractionation is further illustrated in Fig. 1. Clearly, simple stepwise elution with increasing concentrations of sodium chloride and urea resulted in partial fractionation of the different histone species. As judged from scans of the gels, the fraction eluted with 1 *M* sodium chloride contained more than 95% pure Hl, while H2A and H2B accounted for more than 90% of the protein eluted with 2 *M* sodium chloride. The fraction eluted with buffer containing 6.75 *M* urea and 1 *M* sodium chloride consisted of 80–90% H3 and H4, while H2A and H2B amounting to 10–20% of the protein were also present in this fraction.

The different non-histone chromatin protein fractions obtained by chromatography on Blue Sepharose were analysed by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate. It is evident from Fig. 2 that each of the

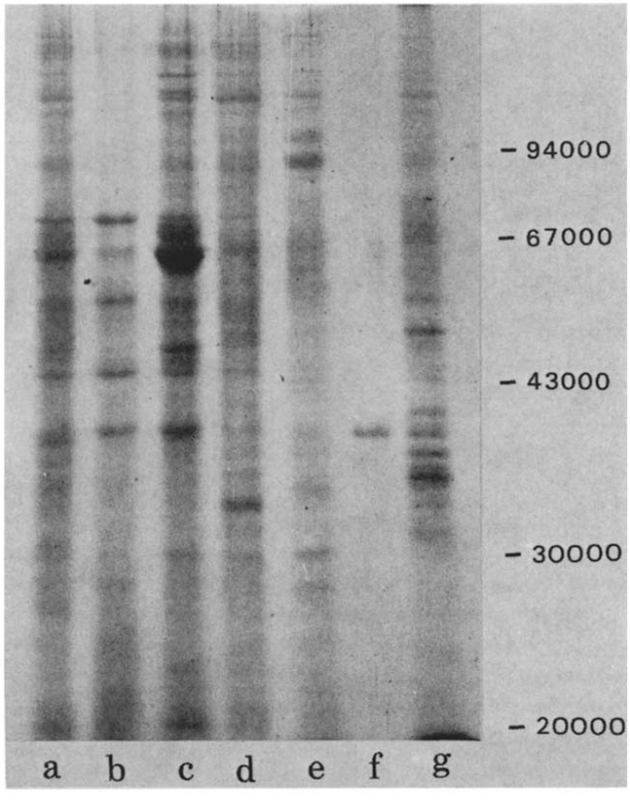

Fig. 2. Polyacrylamide gel electrophoresis of fractions obtained by chromatography of non-histone chromatin proteins on Blue Sepharose. Eluent: 50 m*M* Tris–HCl containing 1 m*M* dithiothreitol and NaCl/urea as specified. (a) Unfractionated non-histones; (b) material not bound with 0.14 *M* NaCl; (c) material retained with 0.14 *M* NaCl, eluted with 0.5 *M* NaCl; (d) material retained with 0.5 *M* NaCl, eluted with 1 *M* NaCl; (e) material retained with 1 *M* NaCl, eluted with 2 *M* NaCl; (f) material retained with 2 *M* NaCl, eluted with 6.75 *M* urea; (g) material retained with 6.75 *M* urea, eluted with 1 *M* NaCl, 6.75 *M* urea. The migration and molecular weights of the standard proteins (Pharmacia LMW Electrophoresis Calibration Kit) are given on the right. Approximately 30 μg of protein were applied to each slot, except for slot (f), where about 10 μg of protein were applied.

fractions contained a variety of different polypeptide chains, with the exception of the fraction obtained by elution with urea, in which one polypeptide with a molecular weight of 38,000 was a major constituent.

To illustrate further the use of Blue Sepharose in the purification of specific chromatin proteins, Fig. 3 shows the results obtained by the chromatography of partially purified poly(ADP–ribose) polymerase. The proteins in this preparation are all retained on DNA–agarose using 0.45 *M* sodium chloride and are eluted using 0.75 *M* sodium chloride[5]; they can therefore be considered to have a high affinity for DNA. We found that all of them also were retained on Blue Sepharose in buffer containing 0.25 *M* sodium chloride. As shown elsewhere[8] (see also Fig. 3b), 90% pure poly(ADP–ribose) polymerase is obtained by an increase in the sodium chloride concentration to 0.4 *M*. Fig. 3c shows that one protein, which we have identified as histone Hl on account of its migration in polyacrylamide gels and its solubility in perchloric acid (results not shown), was eluted with 1 *M* sodium chloride, with a purity of 90%. About half of the proteins still retained could be eluted with 2 *M* sodium chloride, while the remainder demanded a combination of high urea and sodium chloride concentrations for elution to occur. Thus, despite the retention of all of the proteins in the preparation in 0.25 *M* sodium chloride, a substantial purification of two of them was obtained by a careful choice of the elution conditions.

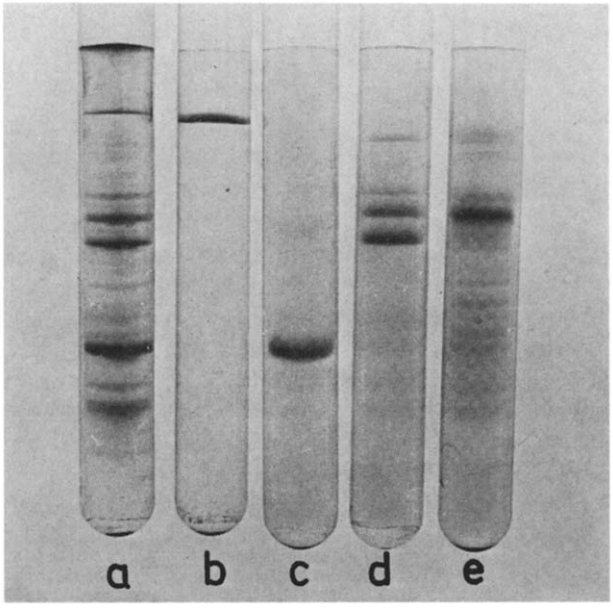

Fig. 3. Polyacrylamide gel electrophoresis of fractions obtained by chromatography of partially purified poly(ADP-ribose) polymerase on Blue Sepharose. Eluent: 10 m*M* Tris–HCl (pH 8.0) containing 10% glycerol, 0.02% NaN_3, 1 m*M* dithiothreitol and NaCl/urea as specified. (a) Unfractionated material; (b) material retained with 0.25 M NaCl, eluted with 0.4 *M* NaCl; (c) material retained with 0.4 *M* NaCl, eluted with 1 *M* NaCl; (d) material retained with 1 *M* NaCl, eluted with 2 *M* NaCl; (e) material retained with 2 *M* NaCl, eluted with 1 *M* NaCl, 6.75 *M* urea. Electrophoresis conditions: see Experimental.

The observation that about 40% of a protein preparation containing proteins with a high affinity for DNA was retained on Blue Sepharose in 2 *M* sodium chloride, while only 8% of unfractionated non-histones was retained to the same degree, suggests some correlation between the strength of the protein–DNA and the protein–Cibacron Blue interactions. This may indicate that the dye interacts with a site on the proteins which normally binds nucleic acids, and that the retention of chromatin proteins on Blue Sepharose reflects their ability to interact with DNA. This conclusion is substantiated by the fact that several other proteins which interact with nucleic acids, such as DNA polymerase[3,13], RNA polymerase[14], ribonuclease[3], polynucleotide phosphorylase[15] and snake venom phosphodiesterase[16], have been reported to be retained on Cibacron Blue–agarose columns.

In conclusion, we have shown that, in contrast to cytoplasmic proteins, most chromatin proteins will bind to Blue Sepharose at a moderate ionic strength. We have demonstrated elsewhere how this binding can be exploited for the purification of the chromatin protein poly(ADP–ribose) polymerase[8]. It seems likely that Blue Sepharose and related matrices may be of use in the purification of other chromatin proteins, especially when taking into account the multitude of possible elution conditions. In the purification of nucleotide-requiring enzymes, for instance, the use of nucleotide-containing eluents and changes in the pH of the eluent have been exploited[1,2]. Thus, we expect that chromatography on Cibacron Blue–agarose columns may prove valuable for the purification and characterization of chromatin proteins.

ACKNOWLEDGEMENT

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