Isolation of the intermediate filament protein vimentin by chromatofocusing

Hans Bloemendal, Mia Willemsen, Gerrit Groenewoud and Paul Oomen

Department of Biochemistry, University of Nijmegen, Geert Grooteplein N21, 6525 EZ Nijmegen, The Netherlands

Received 19 November 1984

A novel, simple and relatively rapid method is described for the isolation of the intermediate-sized filament protein vimentin from eye lens tissue. Chromatofocusing is applied as the sole purification step. The apparent isoelectric point of the protein in 6 M urea and at 22°C is 4.9. Electrophoretic mobility on one- and two-dimensional polyacrylamide gels, solubility in 6 M urea and amino acid composition were used for identification

Vimentin *Intermediate filament protein* *Chromatofocusing*

1. INTRODUCTION

Intermediate filaments (IF) are major constituents of the cytoskeleton of higher eukaryotes [1]. In general IF contain vimentin as protein subunits in cells of mesenchymal origin and in cells grown in culture [2]. Interestingly lens tissue, albeit originating from epithelial cells, produces vimentin both in vivo and in vitro [3]. For this reason lenticular tissue is very convenient for the isolation of this protein. In 1979 Kibbelaar et al. [4] reported the isolation of calf lens vimentin by preparative SDS–polyacrylamide gel electrophoresis (PAGE). Later on Geisler and Weber [5] applied a similar procedure for the isolation of porcine lens vimentin. In both cases rather small yields were obtained. Nelson et al. [6] isolated the protein from Ehrlich ascites cells grown in suspension culture by using DNA-cellulose affinity chromatography.

Here we describe a novel, rapid and very simple method for the isolation of vimentin from lenticular tissue. The only purification step is chromatofocusing [7,8] in 6 M urea [9] at a pH range 5.2–4.5 of the urea-soluble lens fraction.

2. MATERIALS AND METHODS

2.1. *Preparation of lens tissue and sample*

Calf eyes were obtained fresh from the slaughterhouse and transported on ice. The lenses were removed and 3 mm of the outer cortex of each decapsulated lens was isolated by punching with a glass tube of appropriate size. From 60 lenses about 100 g of cortical material was obtained. The latter was stirred in 200 ml of 0.05 M ammonium acetate buffer, pH 7.0 for 1 h. This treatment dissolves the bulk of the water-soluble lens crystallins. After 30 min centrifugation in a Sorvall centrifuge using the SA600 rotor at 12000 rpm the extraction with buffer is repeated twice in 200 ml of distilled water. The supernatants are discarded and the pellet is stirred each time for 45 min. Thereafter the pellet is suspended in 50 ml 0.025 M L-histidine buffer adjusted to pH 7.0 with 0.1 M HCl and containing 6 M urea, stirred for 2 h and again spun in the Sorvall centrifuge at 12000 rpm for 30 min.

The supernatant is adjusted to pH 5.2 with 0.1 M HCl and used as sample solution.

2.2. *Chromatofocusing*

The ion-exchange resin PBE94 and the polybuffer 74 were purchased from Pharmacia, Uppsala, Sweden. A PBE94 column equilibrated in 0.025 M L-histidine buffer containing 6 M urea, pH 5.2 at 22°C was used. The column dimensions were 1 × 60 cm and the bed height after packing 50 cm. All buffers were degassed before use. A 1 cm layer of

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

Sephadex G-25 coarse was applied on top of the equilibrated column. The urea-soluble lens fraction was loaded onto the column. Elution was carried out first with the histidine buffer (pH 5.2) to bind all protein whose isoelectric points are below 5.2. Thereafter the column was developed by elution with polybuffer 74, diluted 1:10, containing 6 M urea and adjusted first to pH 4.8 and thereafter to 4.5 with 0.1 M HCl.

Application of the sample solution was carried out at a pump speed of 36.0 ml/h whereas elution occurred at 13.8 ml/h. The separated fractions were dialyzed against deionized water and lyophilized.

2.3. *PAGE*

One-dimensional SDS gel electrophoresis was carried out on gels containing 13% polyacrylamide according to the method of Laemmli and Faore [10]. Two-dimensional gel electrophoresis was done according to O'Farrell [11].

2.4. *Amino acid composition*

The amino acid content of the purified vimentin was determined on a Chromaspek amino acid analyser. Samples were hydrolyzed for 22 h in 6 M HCl under vacuum at 110°C.

3. RESULTS AND DISCUSSION

Interest in the proteins that compose the intermediate-sized filaments, one of the major structural elements of the cytoskeleton, has been increased considerably during the last few years [12–14]. For a number of purposes, such as structural and evolutionary studies and the preparation of antibodies, the availability of pure IF proteins is a prerequisite.

In our hands chromatofocusing appeared to be an extremely useful procedure for the isolation of pure vimentin, the protein subunit of IF from mesenchymal tissues and most cells grown in culture. As described in section 2 calf eye lens tissue was used as starting material. Vertebrate lenses can be dissolved completely by 3 subsequent solubilization steps [15]. Vimentin and other cytoskeletal proteins are found preferentially in the water-insoluble and urea-soluble fractions (fig.1b,c). The urea-soluble fraction was used as sample solution for chromatofocusing. From earlier protein synthesis experiments, including 2-dimensional gel electrophoresis [16], it was known that vimentin has an isoelectric point below that of the most acidic crystallin subunits (pH 5.2). For this reason the fractionation was carried out with an elution buffer adjusted to pH 4.8 and 4.5, respectively. The separation pattern obtained upon chromatofocusing is shown in fig.2.

Thereafter, aliquots from isolated fractions after dialysis and lyophilization were subjected to SDS–PAGE. As can be seen, pure vimentin is mainly concentrated in 2 fractions of the column eluting at pH 4.98–4.70 (fig.1k,l). For further analysis the protein from these fractions was subjected to 2-dimensional electrophoresis (fig.3B). As expected, both the isoelectric point and the molecular mass were similar to values reported in the literature.

The amino acid content of the protein from those fractions was also determined and compared to the amino acid composition of a preparation purified previously by preparative SDS–PAGE [4], a much more cumbersome method (table 1).

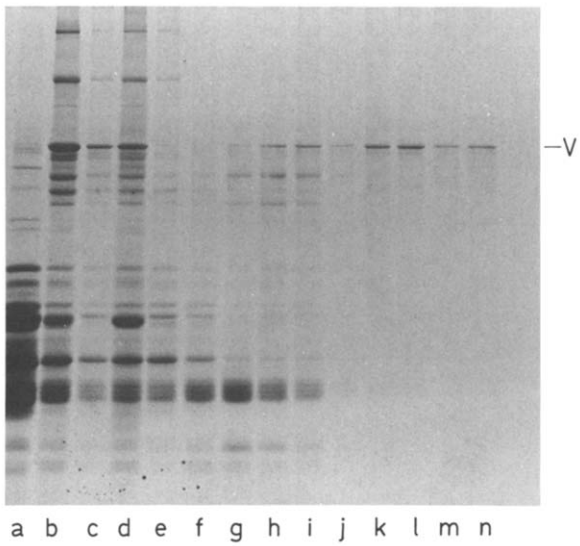

Fig.1. SDS–PAGE of lens protein fraction before and after chromatofocusing. (a) Water-soluble fraction, (b) water-insoluble fraction, (c) urea-soluble fraction, (d) urea-insoluble fraction, (e) fraction not retained by the chromatofocusing column at pH 5.2, (f–n) fractions after developing the chromatofocusing column: f, 1–10; g, 11–16; h, 17–25; i, 26–35; j, 36–60; k, 61–70; l, 71–85; m, 86–99; n, 100–104.

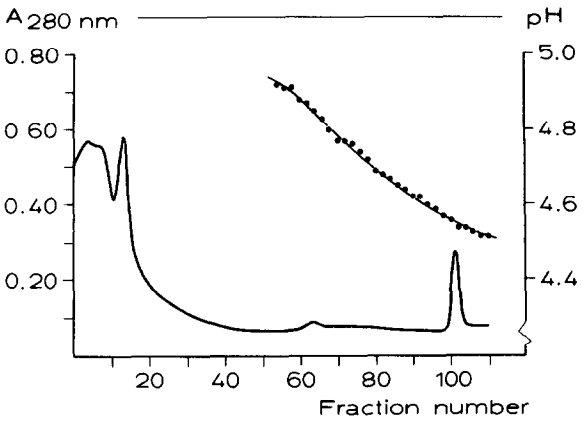

Fig.2. Separation profile of the urea-soluble lens fraction after chromatofocusing between pH 5.2 and 4.5.

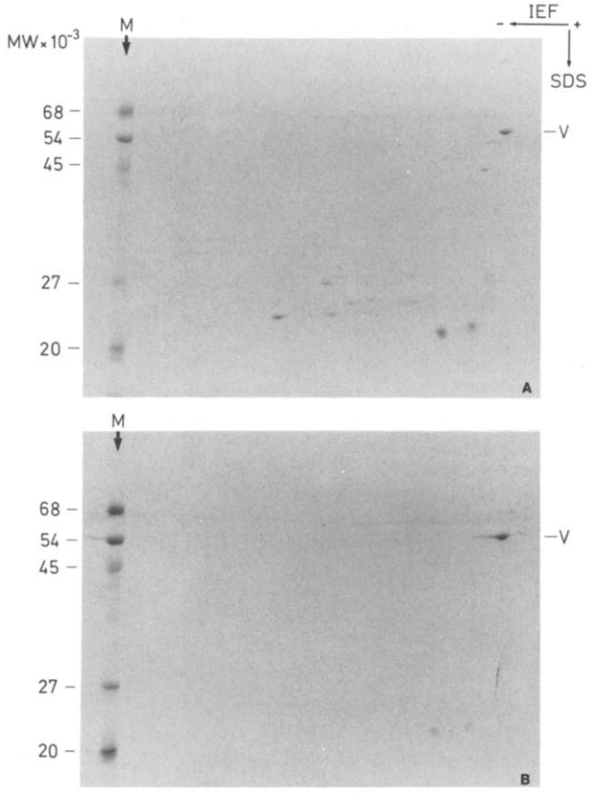

Fig.3. 2-Dimensional gel electrophoresis of the total urea-soluble lens fraction (A) and vimentin isolated after chromatofocusing (B).

Table 1

Comparison of the amino acid composition of calf lens vimentin isolated by chromatofocusing (I) and preparative SDS–PAGE (II)

	I	II	III
Asp + Asn	10.5	9.5	11.6 (6.2 + 5.4)
Thr	5.0	5.5	5.2
Ser	7.3	7.2	9.5
Glu + Gln	16.7	17.5	19.1 (11.8 + 7.3)
Pro	3.2	3.1	1.7
Gly	6.2	5.1	3.0
Ala	8.1	10.4	6.5
Cys	ND	ND	0.2
Val	5.2	4.7	5.2
Met	1.8	1.8	1.9
Ile	3.4	3.5	3.2
Leu	10.5	10.8	12.0
Tyr	2.9	2.6	2.8
Phe	3.2	2.3	2.2
Trp	ND	ND	0.2
His	3.5	2.1	1.3
Lys	5.3	5.7	5.0
Arg	8.0	9.2	9.3

Column III shows the amino acid composition of hamster vimentin as predicted from the coding sequence of the cloned gene [14]. ND, not determined

For additional comparison the amino acid composition predicted from the corresponding nucleotide sequence of the hamster vimentin gene [17] is also given. Whereas there is high agreement between the values of both calf lens preparations there are significant differences at the level of a few amino acids of hamster vimentin (in particular Gly, Pro, Ala). This may be due to evolutionary determined differences. From DNA recombinant studies [18] we knew that there is high homology between hamster vimentin and the same protein from other species. But this homology is restricted to the so-called coiled coil regions and is not found in the NH2- and COOH-terminal extensions [19].

At any rate our method enables easy isolation of the vimentin component present in the urea-soluble fraction of vertebrate lenses.

REFERENCES

[1] Lazarides, E. (1980) Nature 283, 249–256.

[2] Franke, W.W., Schmid, E., Schiller, D.L., Winter, S., Jarasch, E.D., Moll, R., Denk, H., Jackson, B. and Illmensee, K. (1981) Cell 27, 75–84.

[3] Ramaekers, F.C.S. and Bloemendal, H. (1981) in: Molecular and Cellular Biology of the Eye Lens (Bloemendal, H. ed.) pp.85–136, John Wiley & Sons, New York.

[4] Kibbelaar, M.A., Selten-Versteegen, A.-M.E., Dunia, I., Benedetti, E.L. and Bloemendal, H. (1979) Eur. J. Biochem. 95, 543–549.

[5] Geisler, N. and Weber, K. (1981) FEBS Lett. 125, 253–256.

[6] Nelson, W.J., Vorgias, C.E. and Traub, P. (1982) Biochem. Biophys. Res. Commun. 106, 1141–1147.

[7] Sluyterman, L.A.A. and Elgersma, O. (1978) J. Chromatogr. 150, 17–30.

[8] Sluyterman, L.A.A. and Wijdenes, J. (1978) J. Chromatogr. 150, 31–44.

[9] Bloemendal, H. and Groenewoud, G. (1981) Anal. Biochem. 117, 327–329.

[10] Laemmli, U.K. and Faore, M. (1973) J. Mol. Biol. 80, 575–599.

[11] O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007–4021.

[12] Ramaekers, F.C.S., Boomkens, T.R. and Bloemendal, H. (1981) Exp. Cell Res. 135, 454–461.

[13] Geisler, N. and Weber, K. (1981) Proc. Natl. Acad. Sci. USA 78, 4120–4123.

[14] Osborn, M. and Weber, K. (1982) Cell 31, 303–306.

[15] Bloemendal, H. (1977) Science 197, 127–138.

[16] Ramaekers, F.C.S., Dunia, I., Dodemont, H.J., Benedetti, E.L. and Bloemendal, H. (1982) Proc. Natl. Acad. Sci. USA 79, 3208–3212.

[17] Quax, W., Vree Egberts, W., Hendriks, W., Quax-Jeuken, Y. and Bloemendal, H. (1983) Cell 35, 215–223.

[18] Quax, W.J., Dodemont, H.J., Lenstra, J.A., Ramaekers, F.C.S., Soriano, P., Van Workum, M.E.S., Bernardi, G. and Bloemendal, H. (1982) in: Stability and Switching in Cellular Differentiation (Clayton, R.M. and Truman, D.E.S. eds) Plenum, New York, pp.359–366.

[19] Quax-Jeuken, Y.E.F.M., Quax, W.J. and Bloemendal, H. (1983) Proc. Natl. Acad. Sci. USA 80, 3548–3552.