Journal of Chromatography, 570 (1991) 173–179
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5983

Short Communication

High-yield two-step chromatographic procedure for purification of fatty acid-binding protein from human heart

FRANS A. VAN NIEUWENHOVEN*, MICHAËL M. VORK, DON A. M. SURTEL, APPIE H. KLEINE, GER J. VAN DER VUSSE and JAN F. C. GLATZ

Department of Physiology, University of Limburg, P.O. Box 616, 6200 MD Maastricht (Netherlands)

(First received March 12th, 1991; revised manuscript received April 16th, 1991)

ABSTRACT

A high-yield procedure for the purification of cytoplasmic fatty acid-binding protein from human heart (H-FABP) is described. H-FABP was purified by gel permeation chromatography on a Sephacryl S-200 column followed by anion-exchange chromatography on a Sepharose Q fast-flow column at pH 7.0. At this pH H-FABP binds strongly to the column and can be selectively eluted with a salt gradient. The two-step procedure showed a high degree of reproducibility. On average 50 mg of H-FABP was obtained from 150 g of human heart tissue, which corresponds to a recovery of about 50%. Purity was confirmed by gel electrophoresis and isoelectric focusing. Binding of oleic acid to purified H-FABP, using the Lipidex 1000 assay, revealed a maximal binding of 0.75 ± 0.01 mol fatty acid/mol protein and a dissociation constant of 0.19 ± 0.01 μM.

INTRODUCTION

Fatty acid-binding protein (FABP) is a 14–15 kDa cytoplasmic protein abundantly present in the heart and various other tissues. There are three main types of cytoplasmic FABP, named after the sources of their first isolation: liver (L-FABP), intestinal (I-FABP) and heart (H-FABP). FABP is capable of binding fatty acids and is considered to play an important role in fatty acid metabolism [1,2].

In human heart, FABP makes up about 2% of the cytosolic protein mass [3,4]. The purification of human H-FABP as described in literature is a time-consuming process involving at least three steps [4,6] or has reportedly a low recovery [5]. In view of the difficulty of obtaining human material, the availability of a rapid and high-yielding method for purification is quite important.

In this paper we introduce a two-step and high-yielding chromatographic procedure for the purification of human H-FABP. Since no agreement exists on the binding characteristics of human H-FABP [5,6] we also carried out binding stud-

0378-4347/91/$03.50

ies with the Lipidex 1000 assay, as described by Glatz and Veerkamp [7] and modified by Vork *et al.* [8], to monitor the maximum binding (B_{max}) and dissociation constant (K_D) for fatty acid binding.

EXPERIMENTAL

Homogenization of human heart tissue

All steps of the purification procedure were carried out at 4°C. Samples of human heart tissue were obtained by autopsy, and stored at −20°C until use. Human heart tissue, from which visible fat was removed, was homogenized (20%, w/v) in phosphate-buffered saline (PBS), containing 10 m*M* β-mercaptoethanol, using an ultra turrax homogenizer. After centrifugation of the homogenate at 2600 *g* for 10 min the pellet was resuspended in the original volume of buffer and centrifuged again. The combined supernatants were subsequently centrifuged at 105 000 *g* for 90 min. The resulting supernatant was then concentrated by ultrafiltration using a Diaflo YM-5 membrane (Amicon, Danvers, MA, USA).

Gel permeation chromatography

Gel permeation chromatography was carried out on a Sephacryl S-200 SF column (90 cm × 5 cm, Pharmacia-LKB, Uppsala, Sweden) equilibrated with homogenization buffer at a flow-rate of 3 ml/min. The elution was monitored for protein at 280 nm using a UV monitor (UV-1, Pharmacia-LKB). Fractions of 3 min were collected with a fraction collector (2211 Superrac, Pharmacia-LKB). The low-molecular-mass fractions (on average fractions 100–150) were pooled, concentrated and dialysed against 5 m*M* imidazole buffer (pH 7.0), containing 5 m*M* β-mercaptoethanol.

Anion-exchange chromatography

The dialysed and concentrated low-molecular-mass fraction was applied to a Sepharose Q fast-flow anion-exchange column (15 cm × 1.6 cm, Pharmacia-LKB) and eluted at a flow-rate of 2 ml/min. A 5 m*M* imidazole buffer (pH 7.0) containing 5 m*M* β-mercaptoethanol was used as starting buffer. Elution of protein was monitored at 280 nm with a UV monitor (UV-1, Pharmacia-LKB). Fractions of 5 min were collected with a fraction collector (2211 Superrac, Pharmacia-LKB). H-FABP was eluted using a 300-ml linear gradient of 0–20 m*M* sodium chloride in starting buffer. Thereafter residual bound protein was eluted with a 5 m*M* imidazole buffer (pH 7.0), containing 5 m*M* β-mercaptoethanol and 2 *M* sodium chloride. The fractions containing H-FABP were identified by the Lipidex 1000 assay [7] and subsequently collected, concentrated and dialysed against PBS.

Gel permeation chromatography on a Superdex-75 column

To detect any impurities a small portion of the H-FABP fraction produced by

anion-exchange chromatography was applied to a Superdex-75 gel permeation chromatography column (60 cm × 2.6 cm, Pharmacia-LKB) and equilibrated with PBS at a flow-rate of 2 ml/min. The elution was monitored for protein at 280 nm using a UV monitor (UV-1, Pharmacia-LKB). Fractions of 5 min were collected with a fraction collector (2211 Superrac, Pharmacia-LKB).

Electrophoresis and isoelectric focusing

H-FABP-containing fractions at various stages of purification were subjected to sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing, both using the Pharmacia Phast System (Pharmacia-LKB). SDS-PAGE was performed on a Phastgel (gradient 8–25) using SDS buffer strips (Pharmacia-LKB). Isoelectric focusing was carried out on a Phastgel IEF 3-9 (Pharmacia-LKB). Both electrophoresis and isoelectric focusing were performed exactly as dictated in the instruction manual of the Pharmacia Phast System (Pharmacia-LKB).

Fatty acid-binding studies

Binding of [1-^{14}C]oleic acid (specific activity 54.9 Ci/mol; Amersham International, Amersham, UK) to H-FABP was studied with the Lipidex 1000 assay as described by Glatz and Veerkamp [7] and modified by Vork *et al.* [8]. Data obtained from the binding studies were analysed according to Zivin and Waud [9].

Protein concentration

Protein concentrations were determined with the method of Withaker and Granum [10], the validity of which was confirmed by amino acid analyses. To this end protein hydrolysis was carried out with 6 *M* hydrochloric acid for 18 h at 110°C. Subsequently, amino acid analysis was carried out using standard high-performance liquid chromatography (HPLC) techniques according to Van Eijk *et al.* [11].

RESULTS AND DISCUSSION

Purification of H-FABP

Purification was started with 150 g (wet weight) of human heart tissue. After homogenization, aliquots of the supernatant were applied to a Sephacryl S-200 SF gel permeation chromatography column (eight separate runs). The low-molecular-mass fractions of the individual runs were pooled, concentrated and dialysed against starting buffer for anion-exchange chromatography. This fraction was applied to anion-exchange chromatography in three portions. Fig. 1 shows one of the three identical runs on the anion-exchange chromatography column. H-FABP is eluted at approximately 14 m*M* sodium chloride in starting buffer and is almost separated to the baseline. The fatty acid-binding curve shown in Fig. 1

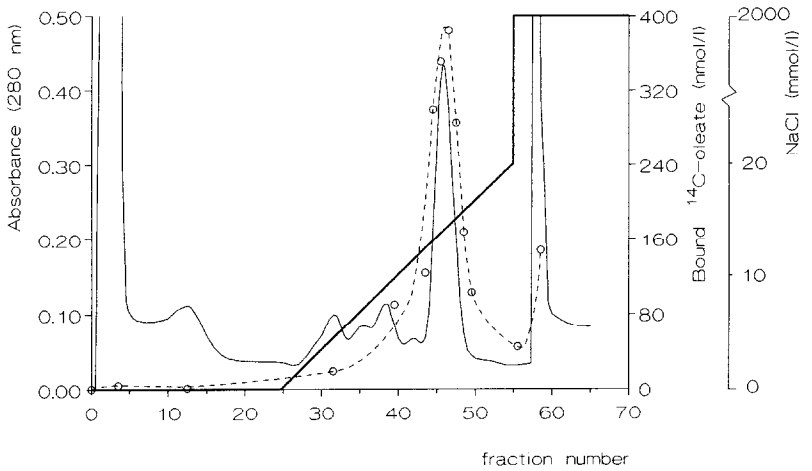

Fig. 1. Anion-exchange chromatography on a Sepharose Q fast-flow column (15 cm × 1.6 cm) of the low-molecular-mass fraction obtained by gel permeation chromatography. H-FABP was eluted with a linear gradient of sodium chloride (bold line) in starting buffer. Elution was monitored at A_{280} (solid line) for protein. Fatty acid binding was measured with the Lipidex assay using [^{14}C]oleate (broken line).

follows the protein curve exactly, suggesting that the H-FABP fraction obtained after the anion-exchange chromatography is pure. Proteins still bound at the end of the linear gradient (20 m*M* sodium chloride) were eluted with 2 *M* sodium chloride. The latter fraction showed some [^{14}C]oleate binding, probably due to residual albumin not removed by gel permeation chromatography. The H-FABP fraction was collected, concentrated and dialysed against PBS.

To confirm purity a small portion of the H-FABP fraction was applied to a Superdex-75 gel permeation chromatography column. This column was calibrated with four proteins in the M_r range of H-FABP. The elution curves of the calibration run and the run with the H-FABP fraction collected from anion-exchange chromatography are presented in Fig. 2A and B, respectively. The elution curve of the H-FABP fraction shows only one symmetrical peak, indicating that the H-FABP obtained after anion-exchange chromatography is pure. The molecular size of H-FABP as derived from Fig. 2 is 15 kDa.

Purity of H-FABP was further confirmed by SDS-PAGE and isoelectric focusing as presented in Fig. 3. This figure also shows that the human H-FABP has an isoelectric point of 5.2.

In our hands 105 000 *g* supernatants prepared from human heart contain of the order of 35 mg cytosolic protein per gram wet weight of tissue. Since H-FABP comprises about 2% of the cytosolic protein mass in human heart [3,4], the H-FABP content of 1 g of human cardiac tissue is about 0.7 mg. The purification procedure as described yielded 50 mg of H-FABP from 150 g of heart tissue, indicating that the overall recovery amounts to about 50%. The purification procedure is highly reproducible as the H-FABP peaks in three separate anion-exchange chromatography runs were eluted at 14 m*M* sodium chloride in all

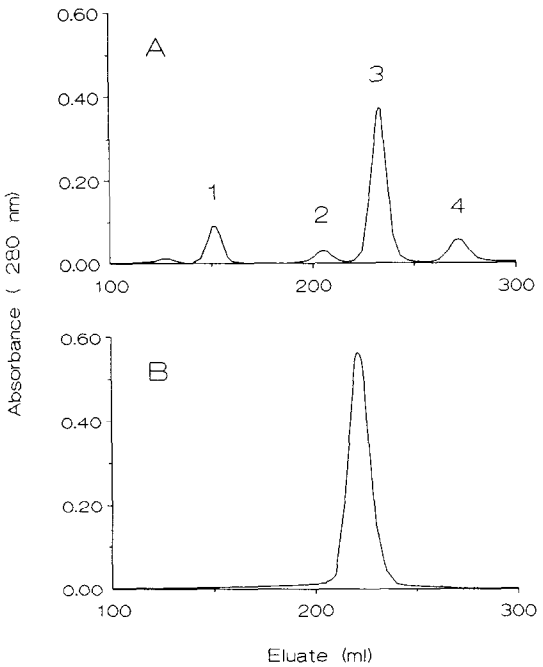

Fig. 2. Gel permeation chromatography on a Superdex-75 column (60 cm × 2.6 cm). (A) Run with molecular mass calibration proteins: 1 = bovine serum albumin, 67 kDa; 2 = trypsin inhibitor, 20.1 kDa; 3 = cytochrome C, 12.5 kDa; 4 = aprotinin, 6.5 kDa. (B) Run with H-FABP previously purified by anion-exchange chromatography.

cases. Furthermore, the two-step purification method, with a small adjustment of the sodium chloride gradient in the anion-exchange chromatography, appeared also to be applicable for the FABPs from rat heart and rabbit heart (unpublished observations), and most likely also for H-FABP originating from other species.

Fatty acid-binding studies

The concentration of the H-FABP solution used for the binding studies was determined by amino acid analysis. The concentration value obtained by this method was slightly higher (10%) than the concentration determined according to Withaker and Granum [10].

Data from the binding studies were analysed according to Zivin and Waud [9] and visualized using an Eadie–Hofstee plot (Fig. 4). The intercept of the y-axis represents the maximal fatty acid binding (B_{max}), while the dissociation constant (K_D) was derived from the slope of the curve. Maximal binding of fatty acid to H-FABP was found to be 0.75 ± 0.01 mol oleate/mol H-FABP, and the dissociation constant K_D was 0.19 ± 0.01 μM. The value of B_{max} is not in accordance with the theoretical binding capacity of 1 mol of fatty acid/mol H-FABP as found for rat heart FABP by Vork *et al.* [8]. A satisfactory explanation for the obvious

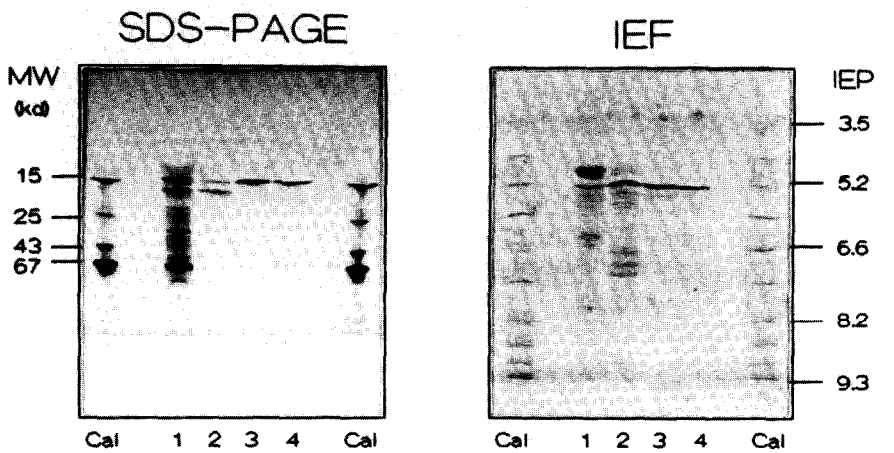

Fig. 3. SDS-PAGE and isoelectric focusing (IEF) illustrating the chromatographic steps of the purification. Lane 1 = sample after homogenization; lane 2 = sample after gel permeation chromatography on Sephacryl S-200; lane 3 = sample after anion-exchange chromatography; lane 4 = sample after gel permeation chromatography on Superdex-75; Cal lane = calibration proteins for SDS-PAGE and IEF. Calibration proteins for SDS-PAGE were: bovine serum albumin, 67 kDa; ovalbumin, 43 kDa; chromotrypsinogen A, 25 kDa; and purified human H-FABP, 15 kDa. Calibration proteins for IEF were: amyloglucosidase, 3.50; soybean trypsin inhibitor, 4.55; β-lactoglobulin A, 5.20; bovine carbonic anhydrase B, 5.85; human carbonic anhydrase B, 6.55; horse myoglobin, 6.85; horse myoglobin, 7.35; lentil lectin, 8.15; lentil lectin, 8.45; lentil lectin, 8.65; and trypsinogen, 9.30.

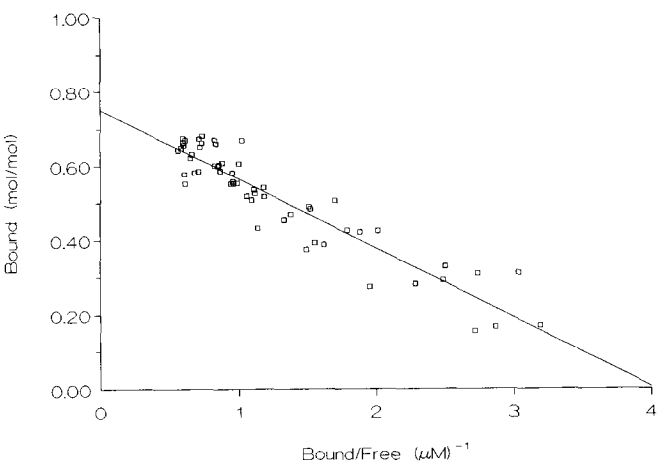

Fig. 4. Eadie–Hofstee plot of the binding of [^{14}C]oleate by human H-FABP. The parameter on the x-axis represents moles of fatty acids bound per mole of H-FABP (mol/mol), and the parameter on the y-axis represents the ratio of the bound fatty acid (mol fatty acid/mol H-FABP) and the concentration of free (unbound) fatty acids (μM). Thus the bound/free ratio is given in μM^{-1}. Maximal oleate binding (B_{max}) = 0.75 ± 0.01 mol fatty acids/mol H-FABP, and the dissociation constant (K_D) = 0.19 ± 0.01 μM.

discrepancy between human and rat cardiac FABP awaits further experimentation.

In conclusion, the described method for the purification of human H-FABP is a fast and simple chromatographic procedure, showing a high recovery. The procedure may also be used for the purification of H-FABP from other species as well as other types of mammalian FABP.

ACKNOWLEDGEMENTS

This work was supported by the Netherlands Heart Foundation Grant 88.073, and by STIPT, Executive Agency for Technology Policy, Grant MTR88002. The authors wish to thank Hans J. M. van Eijk for performing the amino acid analyses and the Department of Pathology (Academic Hospital, Maastricht, Netherlands) for obtaining human heart tissue.

REFERENCES

1 N. M. Bass, *Int. Rev. Cytol.*, 111 (1988) 143–184.
2 J. F. C. Glatz and G. J. Van der Vusse, *Mol. Cell. Biochem.*, 98 (1990) 237–251.
3 R. J. A. Paulussen, H. T. B. Van Moerkerk and J. H. Veerkamp, *Int. J. Biochem.*, 22 (1990) 393–398.
4 C. Unterberg, G. Heidl, D. Von Bassewitz and F. Spener, *J. Lipid. Res.*, 27 (1986) 1287–1293.
5 R. J. A. Paulussen, C. P. E. Van der Logt and J. H. Veerkamp, *Arch. Biochem. Biophys.*, 264 (1988) 533–545.
6 G. D. Offner, P. Brecher, W. B. Sawlivich, C. E. Costello and R. F. Troxler, *Biochem. J.*, 252 (1988) 191–198.
7 J. F. C. Glatz and J. H. Veerkamp, *Anal. Biochem.*, 132 (1983) 89–95.
8 M. M. Vork, J. F. C. Glatz, D. A. M. Surtel and G. J. Van der Vusse, *Mol. Cell. Biochem.*, 98 (1990) 111–117.
9 J. A. Zivin and D. R. Waud, *Life Sci.*, 30 (1982) 1407–1422.
10 J. R. Withaker and P. E. Granum, *Anal. Biochem.*, 109 (1980) 156–159.
11 H. J. M. Van Eijk, M. A. H. Van der Heijden, C. L. H. Van Berlo and P. B. Soeters, *Clin. Chem.*, 34 (1988) 2510–2513.