Studies on fatty acid-binding proteins

Changes in the concentration of hepatic fatty acid-binding protein during development in the rat

Mary SHERIDAN, Trevor C. I. WILKINSON and David C. WILTON*

Department of Biochemistry, University of Southampton, Southampton SO9 3TU, U.K.

The concentration of hepatic fatty acid-binding protein was determined in the livers of rats at various stages of development from foetus to young adult. Fatty acid-binding protein concentrations were determined by quantifying the fluorescence enhancement on the binding of the fluorescent probe 11-(dansylamino)-undecanoic acid. A 20-fold increase in the concentration of the protein was observed between the foetus and adult, and this increase was confirmed by immuno-blotting. No other protein in the 14000- M_r range was observed in the foetus. Possible alternative fatty acid-binding proteins could not be detected in h.p.l.c.-fractionated foetal cytosol by the fluorescence-enhancement method.

INTRODUCTION

Hepatic FABP is a low- M_r cytosolic protein that binds with high affinity fatty acids and their CoA esters and also other organic anions, in particular haem (Glatz & Veerkamp, 1985). Different FABPs have been identified in other tissues, and, in particular, the proteins from intestine and heart have been characterized. Despite considerable literature on the structure and binding properties of the hepatic protein, its precise role in lipid metabolism remains obscure.

We have recently reported a method for the detection and quantification of rat liver FABP based on the fluorescence enhancement of a fatty acid probe on binding to the protein and we have coupled this assay to a rapid gel-permeation h.p.l.c. method for isolating the protein from the cytosolic fraction of rat liver (Wilkinson & Wilton, 1986). In order to gain further insight into the physiological role of the protein, we decided to use the fluorescence method to measure the concentrations of the protein in liver during development. Before the start of this work there were two contradictory pieces of evidence available as to how the concentration of FABP varied in the liver between the foetus and adult. Dempsey *et al.* (1985) have provided evidence to suggest that there is little change during development, on the basis an immunological method for determining the protein, which they term sterol-carrier protein. In contrast, cDNA studies to determine the concentration of mRNA for FABP during development have identified a significant rise in mRNA concentrations between foetus and adult (Gordon *et al.*, 1985). Such a rise in mRNA concentrations for FABP would normally reflect a change in protein concentrations, since there are very few examples of the translational control of protein synthesis in eukaryotes.

We now report our studies on the concentration of FABP in foetal, neonatal and adult rat liver using the recently developed fluorescence-enhancement method for quantifying the protein (Wilkinson & Wilton, 1986), which is supported by immunological studies using Western blotting. Our results show a dramatic rise in the concentration of this protein during development from a

very low value in the foetus to the normal high value in the adult. Since the completion of this work, it has been reported that the total non-albumin fatty acid-binding capacity of liver cytosols (normally assumed to be FABP) does not alter between the neonatal animal and adult (Paulussen *et al.*, 1986).

MATERIALS AND METHODS

Chemicals

11-(Dansylamino)undecanoic acid was obtained from Molecular Probes, Junction City, OR, U.S.A. The biotin/streptavidin/peroxidase system for Western blotting was supplied by Amersham International, Amersham, Bucks., U.K.

Rat liver preparations

Wistar albino rats were used for the development studies, with female rats being selected after birth. Rats were fed *ad libitum* on a pelleted diet [Labsure CRM(X); K. and K. Greeff, Croydon, Surrey, U.K.] containing (w/w) 18.5% protein, 56% carbohydrate and 3.1% fat. Foetal material (18 days gestation) was obtained by combining at least ten livers. Cytosols and purified FABP were prepared as described previously (Wilkinson & Wilton, 1986).

Detection and measurement of FABP

The fluorescence method for the detection and quantification of FABP in rat liver cytosols has been described previously (Wilkinson & Wilton, 1986). After h.p.l.c., the fractions containing FABP were combined and samples were titrated with 11-(dansylamino)undecanoic acid to obtain the maximum fluorescence enhancement. This enhancement was calibrated from a standard curve with pure FABP. The volume of the sample taken from the combined h.p.l.c. fractions was adjusted so that the total amount of FABP in the fluorescence assay did not exceed 2 μ g.

Evaluation of cytosols (0.6 ml) by normal gel filtration was achieved with a Sephadex G-75 column (0.75 cm $\times$ 50 cm) equilibrated with 10 mM-potassium

Abbreviation used: FABP, fatty acid-binding protein.

* To whom correspondence should be addressed.

Fig. 1. H.p.l.c. analysis of rat liver cytosol from foetal and 60-day-old rats and location of FABP by using 11-(dansylamino)undecanoic acid fluorescence enhancement

(a) and (b) H.p.l.c. analysis of rat liver cytosol fraction: 100 μ l of cytosol was run on a TSK-G 2000 SW column (7.5 mm $\times$ 600 mm). (a) 60-day-old female rat liver cytosol (20.5 mg of protein/ml); (b) foetal rat liver cytosol (18.2 mg of protein/ml). (c) and (d) Fractions (1 ml) were collected during the h.p.l.c. analysis; 0.5 ml samples were made up to 1 ml, and 10 μ l of 0.1 mM-11-(dansylamino)undecanoic acid was added to each. The fluorescence due to buffer alone has been subtracted. (c) 60-day-old female liver cytosol; (d) foetal cytosol.

phosphate buffer, pH 7.4. Column fractions were analysed by SDS/polyacrylamide-gel electrophoresis (15% acrylamide gel; Laemmli, 1970).

Western blotting was performed according to the method of Burnette (1981) with modifications. Proteins were separated by electrophoresis on SDS/polyacrylamide gels (15% acrylamide), then were electrophoretically transferred to a nitrocellulose membrane (0.2 μ m) by using an electrophoretic transfer buffer containing 25 mM-Tris base, 192 mM-glycine and 20% (v/v) methanol, pH 8.3. A 5% (w/v) bovine serum albumin solution in phosphate-buffered saline (2.7 mM-KCl/0.26 mM- KH_2PO_4 /8.3 mM- Na_2HPO_4 buffer, pH 7.4) was used as the blocking solution and antibody incubation buffer. An antibody dilution of 1:100 was used for all incubations. Filter-bound antibody-antigen complexes were detected by using the biotin/streptavidin/peroxidase system (Amersham) according to the manufacturer's instructions.

Monospecific antiserum to rat liver FABP was produced in male New Zealand White rabbits and was processed to produce a partially purified IgG fraction (Johnstone & Thorpe, 1982).

RESULTS

The livers of rats from various stages of development were homogenized and the cytosolic fraction was prepared by differential centrifugation. Samples of cytosol were subjected to gel-permeation h.p.l.c., and the fractions corresponding to authentic FABP were combined and samples were titrated with the fluorescent fatty acid probe 11-(dansylamino)undecanoic acid (Wilkinson & Wilton, 1986). The fluorescence enhancement achieved on saturation with the probe was compared with a calibration curve for pure hepatic FABP, and the FABP content of the cytosol was determined (Wilkinson & Wilton, 1986). Fig. 1 shows the elution and fluorescence profile after h.p.l.c. for foetal and adult samples. The quantification of FABP for various ages of rat was performed, both in terms of μ g of FABP/mg of cytosolic protein and mg of FABP/g wet wt. of liver, and the results are shown in Fig. 2.

The quantitative results of Fig. 2 confirm the empirical observations of Fig. 1 and clearly show the dramatic rise in FABP content from very low values in the foetus to the high values associated with the adult.

Fig. 2. Measurement of the FABP concentration in rat liver during development

Cytosols were separated by h.p.l.c., and samples of the FABP fraction were titrated with 11-(dansylamino)undecanoic acid and quantified by using 11-(dansylamino)undecanoic acid fluorescence enhancement. Values are the means $\pm$ S.D. At least three different cytosolic preparations were used for each age. (a) FABP concentration expressed as $\mu\text{g/mg}$ of cytosolic protein; (b) FABP concentration expressed as mg/g wet wt. of liver.

Fig. 3. SDS/polyacrylamide-gel-electrophoretic analysis of the 14000- M_r region after separation of rat liver cytosols on a Sephadex G-75 column

A 0.6 ml portion of cytosolic fraction (3.3 mg of protein) of rat liver from foetal, 5-day-old or 60-day-old rats was applied to the column, and the fractions corresponding to the FABP region (fractions 16–19) were analysed by SDS/polyacrylamide-gel electrophoresis and stained with Coomassie Blue.

The 14000- M_r FABP can be readily detectable in adult cytosol after SDS/polyacrylamide-gel electrophoresis; however, the situation for foetal cytosol is obscured by the presence in this region of an intensely staining double band corresponding to haemoglobin subunits due to the fact that the liver is a major haemopoietic organ in the foetus (results not shown).

In order to confirm the very low concentrations of FABP in the foetus and neonatal animal, the 14000- M_r region obtained after normal gel filtration on Sephadex G-75 of liver cytosols was examined by SDS/polyacrylamide-gel electrophoresis. The very low concentration of native 14000- M_r material in the foetus was confirmed (Fig. 3).

Finally, Western blotting was performed after separation of cytosolic fractions of liver on SDS/polyacrylamide-gel electrophoresis, and the material reacting with antibody to pure rat liver FABP was detected by using the biotin/streptavidin/peroxidase system. Although this technique was used in a semi-quantitative manner, it clearly demonstrates the difference in concentration of FABP during development, thus confirming the results of the fluorescence assay (Fig. 4). Also noted is an

apparent aggregation of pure FABP to an apparently covalently linked higher- M_r aggregate when samples of pure protein that had been stored for long periods were subjected to Western-blotting analysis. This high- M_r aggregate was not present in freshly prepared samples of pure FABP, nor is it detected in the cytosol.

DISCUSSION

The 14000- M_r FABP characterized from adult rat liver appears to be present only at very low concentrations in the foetus but increases during postnatal development. This difference must presumably reflect a different physiological role of foetal liver as compared with adult liver in fatty acid metabolism.

Studies on lipid metabolism in liver during development have identified moderate rates of lipogenesis in the foetus which are dramatically inhibited during the suckling period but rise again to high values in the adult (Ballard & Hanson, 1967; Taylor *et al.*, 1967). Therefore it is difficult to correlate the gradual increase in FABP concentration with these changes in lipid metabolism. In view of the major role played by the endocrine system in

Fig. 4. Western blots of cytosols from liver of rats at different ages of development

Samples of cytosol (50 μ g of protein) were subjected to SDS/polyacrylamide-gel electrophoresis (15% acrylamide gel) followed by immuno-blotting with a biotin/streptavidin/peroxidase detection system. The sample of pure FABP that was run as a reference shows a high- M_r aggregate not present in the sample when initially purified. The positions of M_r standards on the original gels are indicated.

the regulation of lipid metabolism, hormonal changes during development may provide the stimulus for increased FABP synthesis. It is noteworthy, therefore, that two hormones whose concentrations rise during development, namely thyroxine and the glucocorticoids (Henning, 1981), have been implicated as inducers of FABP in hepatocytes (Hargis *et al.*, 1986) in conjunction with insulin.

Our results differ from those reported by Paulussen *et al.* (1986). These authors measured total fatty acid-binding capacity in dealbuminized delipidated cytosol samples and found no developmental changes in this parameter. Although in the adult rat the total non-albumin binding activity is probably accounted for by FABP, the situation for the foetus and neonatal animal may be very different. For example, the foetus produces large amounts of α -foetoprotein, which also has a high affinity for fatty acids, and the production of this protein by the liver continues for several days after birth (Ruoslahti & Seppala, 1979). Although this protein is homologous to albumin, it has been shown only to cross-react with anti-albumin antibodies in the denatured state (Ruoslahti & Seppala, 1979) and hence was probably not removed by the procedure used by Paulussen *et al.* (1986). In addition, the presence of other types of proteins that bind fatty acids may be present in the foetus that are not detected by our fluorescence method. It is noteworthy that 11-(dansylamino)-undecanoic acid will not detect FABP from rat heart (M. Sheridan, T. C. I. Wilkinson & D. C. Wilton, unpublished work), presumably owing to differences in the fatty acid-binding site on this protein.

In conclusion, the very low concentration of hepatic FABP in foetal and neonatal rat liver must reflect the physiological requirement for FABP at these stages of development. Presumably the increasing concentration

of FABP during development must parallel the increasing importance of the liver in fatty acid metabolism, particularly in terms of triacylglycerol synthesis and release.

We thank the Medical Research Council and the Science and Engineering Research Council for Studentships awarded to M.S. and T.C.I.W. respectively.

REFERENCES

- Ballard, F. J. & Hanson, R. W. (1967) *Biochem. J.* **102**, 952–958
- Burnette, W. N. (1981) *Anal. Biochem.* **112**, 195–203
- Dempsey, M. E., Hargis, P. S., McGuire, D. M., McMahon, A., Olson, C. D., Salati, L. M., Clarke, S. D. & Towle, H. C. (1985) *Chem. Phys. Lipids* **38**, 223–237
- Glatz, J. F. C. & Veerkamp, J. H. (1985) *Int. J. Biochem.* **17**, 13–22
- Gordon, J. L., Elshourbagy, N., Lowe, J. B., Lias, W. S., Alpers, D. H. & Taylor, J. M. (1985) *J. Biol. Chem.* **260**, 1995–1998
- Hargis, P. S., Olson, C. D., Clarke, S. D. & Dempsey, M. E. (1986) *J. Biol. Chem.* **261**, 1988–1991
- Henning, S. J. (1981) *Am. J. Physiol.* **241**, G199–G214
- Johnstone, A. & Thorpe, R. (1982) in *Immunochemistry in Practice*, pp. 44–45, Blackwell Scientific Publications, Oxford
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Paulussen, R. J. A., Jansen, G. P. M. & Veerkamp, J. H. (1986) *Biochim. Biophys. Acta* **877**, 342–349
- Ruoslahti, E. & Seppala, M. (1979) *Adv. Cancer Res.* **29**, 275–346
- Taylor, C. B., Bailey, E. & Bartley, W. (1967) *Biochem. J.* **105**, 717–722
- Wilkinson, T. C. I. & Wilton, D. C. (1986) *Biochem. J.* **238**, 419–424