ABSTRACT

Cytosol obtained from differential centrifugation of homogenates from liver and small intestine mucosa was incubated with 1-[14C]oleic acid or 1-[14C]palmitic acid and filtered through Sephadex G-75. Elution profiles for both tissues showed radioactivity in two main peaks, the first corresponding to binding of fatty acid to high molecular weight proteins and the second to a protein fraction with a molecular weight of approximately 12,000 daltons. The low molecular weight fraction had high fatty acid-binding activity, which was greater for oleic than palmitic acid. The findings demonstrate the presence of fatty acid-binding protein in liver and intestinal mucosa of the preruminant calf.

INTRODUCTION

Substantial evidence has been presented by several laboratories that fatty acid-binding protein (FABP) is involved in the uptake, transport, and esterification of long-chain fatty acids in small intestine and liver (1, 13, 16, 18, 21). Most work on the physiological role and properties of FABP has been with rat liver and small intestine, but FABP is present in other tissues of the rat and in many other animal species. For example, FABP has been found in the cytosol of liver, heart, kidney, adipose, and intestinal mucosa of the rat and human (15, 16, 18); liver of the mouse and guinea pig (9); and intestinal mucosa of the newly hatched chick and adult cockerel (8). It appears that FABP in these various tissues comprises closely similar low molecular weight cytosolic proteins (approximately 12,000 daltons), having a high affinity for long-chain fatty acids. They are similar, if not identical, to the Z protein originally described by (12) and (14) and to the aminoazodye-binding protein A of (10).

The present experiments were conducted to ascertain whether FABP is present in the liver and intestinal mucosa of the preruminant dairy calf. Liquid diets, such as milk or milk replacer, are the main source of nutrients for the calf up to weaning. Tallow and lard, the fats commonly used in milk replacers, contain high concentrations of long-chain fatty acids, and it was considered that FABP might have an important function in the absorption and subsequent metabolism of these fatty acids in the calf.

MATERIALS AND METHODS

Materials

Sephadex G-75, G-25 gel filtration equipment and the calibration kit for low molecular weight proteins were purchased from Pharmacia (Canada) Ltd., Dorval, Quebec, Canada. Apparatus and membranes used in concentration of protein solutions were obtained from Amicon Corporation, Lexington, MA. Other suppliers were Amersham (Oakville, Ontario, Canada) for 1-[14C]oleic acid (56.75 mCi/mm mol); New England Nuclear (Lachine, Quebec, Canada) for 1-[14C]palmitic acid (12.5 mCi/mm mol) and Bray’s solution; K and K Laboratories, Inc. (Plainview, NY) for α-bromopalmitic acid; and Sigma Chemical Company (St. Louis, MO) for cytochrome C (horse heart, Type 3).

Source of Tissues

Mucosa samples from liver and small intestine were obtained from four male Holstein calves, 31 d of age. Three calves were used for all work except inhibition of FABP binding of oleic acid by α-bromopalmitate where liver from the fourth calf was used. Calves were fed colostrum for the first 3 d postpartum followed by milk.
replacer for 28 d. Composition of the milk replacer was as reported previously in (5) for control skim milk diet and in general contained (in the dry matter) 69.0% skim milk powder, 8.8% sweet whey, 16.6% beef tallow, 2.0% coconut oil, 1.0% emulsifiers, and 2.6% added minerals and vitamins.

Methods

Liver and the first one-half section of the small intestine after the abomasum were quickly removed and rinsed with phosphate-potassium chloride (KCl) buffer (.01 M phosphate buffer in .154 M KCl, pH 7.4, 4°C). Liver was perfused from a syringe with phosphate-KCl buffer pH 7.4 to remove blood. Liver and intestinal mucosa each were homogenized in 3 vol of phosphate-KCl buffer pH 7.4 using a hand-operated, Teflon-glass Potter-Elvehjem tissue homogenizer, and centrifuged at 10,000 x g for 20 min to remove gross fat, cellular debris, and mitochondria. The low speed infranatant was recentrifuged in a Beckman preparative ultracentrifuge (model L-2) at 105,000 x g for 90 min, 4°C.

Four milliliters of cytosol were incubated with 50 nmol of either 1-[14C] oleic acid (2.82 μCi) or 1-[14C] palmitic acid (.625 μCi) at 37°C for 10 min. Two milliliters of the incubated sample were applied to a column of Sephadex G-75 (2.6 x 46 cm) and separation of proteins achieved by upward flow elution using phosphate-KCl buffer pH 7.4 with a flow rate of 18 ml/h. Three-milliliter fractions of eluate were collected with protein concentration monitored at 280 nm (Bausch and Lomb Spectronic 21) and determined by a modification of the Lowry method (6). Radioactivity in the 3-ml eluate fractions was measured on 1.0-ml aliquots added to 12 ml of Bray's solution (New England Nuclear) in scintillation vials using a Packard Tri-Carb Scintillation Counter (Model 3320).

An estimation of the molecular weight of FABP was obtained by comparing its relative elution volume on Sephadex G-75 with that of proteins of known molecular weight. The Pharmacia gel filtration kit for low molecular weight proteins was used (ribonuclease, chymotrypsinogen A, ovalbumin, bovine serum albumin) and cytochrome C.

Liver FABP fraction was obtained, without prior incubation with 1-[14C]-labeled fatty acids, by monitoring (280 nm) appropriate eluate from liver cytosol filtered on Sephadex G-75 column. To study the effect of α-bromopalmitate on FABP binding of oleic acid, 1-[14C]-labeled oleic acid (10 nmol in 8 μl methyl ethyl ketone) ± α-bromopalmitate (10 nmol in 10 μl methylethyl ketone) was incubated with concentrated FABP fraction (approximately .5 mg protein in 1.0 ml phosphate-KCl buffer; Amicon ultrafiltration UM-2 membrane) for 10 min at 37°C and passed through a Sephadex G-25 column (.9 x 21 cm, 4°C). Fatty acid binding was calculated from radioactivity eluting with the void volume.

Data were analyzed statistically by analysis of variance and t test to determine significance of differences between means (20).

RESULTS AND DISCUSSION

Figure 1 shows a typical elution profile of 1-[14C] oleic acid bound to calf liver cytosolic proteins, when liver cytosol was mixed with 1-[14C] oleic acid and subjected to gel filtration with Sephadex G-75. Results were similar with hepatic cytosol from two other calves. Two major oleic acid-binding peaks were detected. The first radioactivity peak corresponded to high molecular weight proteins that were eluted in the void volume and shortly thereafter. Albumin (67,000 daltons), if present, would have eluted mostly in fraction 31 in the right shoulder of this first peak. The second peak represented a fraction of low molecular weight proteins.

For three calf livers, an average of 18.3 ± .8% (standard error)2 of the 1-[14C] oleic acid applied to the column was recovered in the eluates. The unrecovered fatty acid was bound to the Sephadex. Similar amounts of the free fatty acid added to the column were completely bound to the Sephadex. Protein recoveries here and for other incubations with oleic and palmitic acids with liver and mucosa cytosol showed that protein was not precipitated on the column, bound or unbound to labeled fatty acid. The results are similar to those of Ockner.

2Unless indicated otherwise, all data presented are the mean ± standard error for liver or intestinal mucosa from three calves, one determination each.
Figure 1. Sephadex G-75 chromatography of calf liver cytoplasmic proteins in presence of 1-\[^{14}\text{C}\] oleic acid (upper) or 1-\[^{14}\text{C}\] palmitic acid (lower). Liver cytoplasm (29.4 mg protein with 1.41 gCi oleate, 34.3 mg protein with 0.3125 µCi palmitate) in 2 ml potassium chloride-phosphate buffer, pH 7.4, with 25 nmol \[^{14}\text{C}\] -labeled fatty acid in 10 µl methyl-ethyl ketone, chromatographed on Sephadex G-75 (2.6 X 46 cm, 4°C, 18 ml/h, 3-ml fractions).

and Manning (14), who incubated 1-\[^{14}\text{C}\] oleic acid with rat tissues and recovered about 21% of applied radioactivity from Sephadex G-75 column. Here, of the eluted radioactivity, 30.4 ± 3% was associated with peak 1. The second peak, contained 57.7 ± 4.4% of the eluted radioactivity. Oleic-binding activity for the low molecular weight proteins in the second peak (59,612 ± 2,649 dpm/mg protein) was considerably higher than for the high molecular weight proteins in peak 1 (11,076 ± 379 dpm/mg protein). These observations are comparable with those obtained in similar work with rat liver cytosol by Gloster and Harris (2), who found high oleic acid-binding activity for a low molecular weight protein fraction (12,000 to 13,000 daltons) and much lower activity for a high molecular weight fraction and for albumin.

An estimation of the molecular weight of proteins in peak 2 was obtained by comparing their relative elution volume on Sephadex G-75 with those of standard proteins of known molecular weight. An average molecular weight of 12,100 ± 352 daltons was obtained for six determinations (livers from three calves, two determinations each). This value corresponds closely to the molecular weight reported for FABP in cytosol of rat liver (16) and intestinal mucosa (15), rat and human adipose tissue (7), mouse and guinea pig liver (9), and chicken intestinal mucosa (8). Results obtained here are consistent with the presence of FABP in calf liver cytosol.

Haunerland et al. (4) reported finding two species of FABP (11,800 ± 1,000 daltons) in bovine liver cytosol. Age, sex, and stage of development of the animal were not reported.

Several workers have shown that α-bromopalmitate and flavaspidic acid compete with long-chain fatty acids for binding to FABP from rat intestinal mucosa (16) and adipose (7). Similar in vitro inhibition of oleic binding by calf hepatic FABP was obtained in this study for α-bromopalmitate. The FABP fraction (0.5 mg protein) prepared by Sephadex G-75 filtration of liver cytosol was incubated with 1-\[^{14}\text{C}\] oleic acid (10 nmol) and passed through Sephadex G-25. Fatty acid binding to FABP was calculated from the radioactivity that eluted in the void volume. Binding of oleic acid was 27% lower (P<.05) in the presence of α-bromopalmitate (10 nmol). In triplicate runs for one calf liver, binding of oleate by FABP in the absence of inhibitor was 19.1 ± 1.4% and with α-bromopalmitate binding was 13.9 ± 1.0%. Ockner and Manning (16) have shown that α-bromopalmitate inhibition of FABP binding of oleic acid is reversed with higher oleate intakes.

When 1-\[^{14}\text{C}\] palmitic acid was incubated with liver cytosol and filtered through Sephadex
G-75, palmitate was bound to protein in the same two major peaks encountered with oleic acid (Figure 1), but a lower proportion of the incubated fatty acid was bound. The 1-[¹⁴C]palmitic acid recovered in the eluates was 11.0 ± 9% of that applied to the column. About 80% of the labeled palmitate was bound to the Sephadex and the remainder (free 1-[¹⁴C]palmitic acid) eluted (tubes 80 to 85) substantially after the last of the FABP collected (in tubes 60 to 65). Free palmitic acid added to the column was only partially bound to the Sephadex, the unbound portion being collected in tubes 80 to 85. An average of 36.2 ± 5.1% of the eluted palmitate radioactivity was associated with proteins in the FABP peak. Nanomoles of palmitate bound per milligram protein in FABP fraction was one-third that obtained for oleate. This agrees with reports of several workers that FABP (15, 18) and Z protein (13) have a strong affinity for unsaturated long-chain fatty acids and, to a lesser extent, saturated long-chain fatty acids.

Similar experiments were carried out with calf mucosa cytosol incubated with 1-[¹⁴C]labeled oleic or palmitic acid, and then filtered through Sephadex G-75. As with liver cytosol, oleic acid-binding in the first peak corresponded to high molecular weight proteins eluted in the void volume and slightly later (Figure 2 shows a typical elution profile). However, with mucosa a small peak also appeared after peak 1 where albumin marker compound eluted. Also, as with liver cytosol, a high proportion (52.9 ± 3.8%) of eluted oleic radioactivity was associated with FABP in the second major peak; these FABP had a molecular weight of 12,300 ± 543 daltons. This agrees closely with results from a similar experiment by (15) with 1-[¹⁴C]-oleic acid and rat mucosa cytosol in which 47% of the recovered radioactivity was with the FABP peak. Oleic acid binding (nanomoles oleic acid per milligram protein) by our FABP preparation from mucosa cytosol was about 60% that obtained for liver FABP. Mishkin et al. (14) also have reported a greater binding of oleic acid to Z protein derived from liver than intestinal mucosa.

Very little 1-[¹⁴C] palmitic acid was bound by calf mucosa cytoplasmic proteins (Figure 2). Two small binding peaks were obtained, roughly equivalent to the high and low molecular weight protein peaks obtained for 1-[¹⁴C] oleic acid incubated with mucosa cytosol.

In summary, FABP is present in liver and in small intestine mucosa of the preruminant calf. There is considerable evidence that FABP plays an important role in rats in cellular uptake, transport, and utilization of long-chain fatty acids in liver and small intestine. There are indications from in vitro work that FABP also stimulates several enzymic reactions involved in fatty acid metabolism [reviewed in (9, 17)]. Whether any of these functions of FABP occur in the preruminant calf is not known but is of interest as the calf normally consumes relatively

![Figure 2](https://example.com/f2.png)

**Figure 2.** Gel filtration of calf intestinal mucosa cytoplasmic proteins in presence of 1-[¹⁴C]olate acid (upper) or 1-[¹⁴C] palmitic acid (lower). The [¹⁴C]-labeled fatty acid (25 nmol in 10 µl methyl-ethyl ketone) added to calf mucosal cytoplasm (29.8 mg protein for 1.41 µCi oleate, 29.2 mg protein for 3125 µCi palmitate) in 2 ml potassium chloride-phosphate buffer, pH 7.4, and chromatographed on Sephadex G-75 (2.6 X 46 cm, 4°C, 18 ml/h, 3-ml fractions).
large quantities of long-chain fatty acids in liquid diets before being weaned onto solid ration. It also would be of interest to determine whether FABP has an important role in fatty acid absorption and metabolism in the adult ruminant. In animals with functioning rumens, extensive hydrolysis of esterified lipid occurs in the rumen from microbial lipase action. It also would be of interest to determine whether FABP has an important role in fatty acid absorption and metabolism in the adult ruminant. In animals with functioning rumens, extensive hydrolysis of esterified lipid occurs in the rumen from microbial lipase action. Consequently, the major lipid class in digesta entering the small intestine is unesterified fatty acids, and these are solubilized by lyssolecithin formed in the intestinal lumen from bile-secreted lecithin. This contrasts with the major involvement of monoglycerides in fatty acid absorption in simple stomach animals. Thus, in the mature ruminant, the relatively large amounts of unesterified long-chain fatty acids in contents of small intestine may require mucosal FABP for effective fatty acid absorption and utilization.

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REFERENCES


