# A FATTY ACID BINDING PEPTIDE OF RAT LIVER CYTOSOL

## Characterization and origin

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## 1. Introduction

Three fatty acid binding fractions (FABF) have been found in rat liver cytosol after separation on Sephadex G-75 gel. Knowledge of the chemical nature, the intracellular origin and the role in intracellular lipid metabolism of these 3 fractions differed widely. It was suggested that the main protein in the fraction, with the highest molecular weight, (fraction 1) was albumin whose ability to bind fatty acids (FA) is well known [1–3]. In 1969 the existence of a specific FABF of cytosol the so-called 'Z-protein' (mol. wt 10 000) was first described [4–6]. This 'Z-protein' is the main binding component of the second peak ('Z-fraction'). It is important in binding unsaturated FA [7,10] and increased the acylation of 1,2-diacyl-*sn*-glycerol [8] in in vitro studies. The existence of a third FABF (mol. wt 1500) was described but not characterized [9]. Therefore its chemical nature and physiological function is unknown. We have been interested in the question whether the low molecular weight FABF (peak 3) represents an artifact originating from degradation of one of the other FABF or is a new FA-binding cytosolic component. The present investigation was undertaken with the aim of obtaining further insight into the cellular regulation of lipid biosynthesis. The present report provides evidence that the peak 3 fraction is a peptide. It is not an artifactual product of one of the other FABF. It seems to be a component of microsomes and mitochondria and not a fraction existing physiologically in the cytosol.

## 2. Methods

Preparation of cytosol, gel filtration on Sephadex G-75 superfine, column electrofocusing and measurement of radioactivity were carried out as in [10].

### 2.1. *SDS–Polyacrylamide gel electrophoresis*

Electrophoresis of fractions 1, 2 and 3 was carried out according to [11] in a 12% gel, 1% SDS with a vertical electrophoresis chamber (Desaga, Heidelberg). The peak 3 fraction was delipidated according to [12] before electrophoresis was started.

### 2.2. *Determination of carbohydrates*

This was carried out as described in the following references: glucose with glucose oxidase [13], galactose with galactose dehydrogenase [14], hexosamine [15] and neuraminic acid [16]. Protein was determined by the procedure in [17].

### 2.3. *Preparation of mitochondria and microsomes*

Rat livers were sliced and homogenized in 0.25 M sucrose, 0.001 M EDTA at 4°C with a Potter-type homogenizer (1 g liver/5 ml sucrose solution). The homogenate was centrifuged at 500 × *g* for 10 min, the supernatant was centrifuged at 12 000 × *g* for 20 min. The sediment (mitochondrial) was washed with the homogenisation solution. The 12 000 × *g* supernatant was centrifuged at 105 000 × *g* for 90 min. The sediment of microsomes was also washed.

### 2.4. *Preparation of 'pseudo-cytosol' of microsomes and mitochondria*

The mitochondria or microsomes were resuspended in the homogenisation solution (1:5, w/v) and the suspensions frozen and thawed 3 times, they were then centrifuged at 105 000 × *g* for 90 min. The sediments obtained were incubated with 0.5% SDS in homogenisation solution for 30 min followed by centrifugation at 105 000 × *g* for 90 min. The 105 000 × *g* supernatants were named 'pseudo-cytosol' and labelled with [$^3$H]palmitic acid as described for cytosol [10]. The labelled pseudo-cytosols were separated on Sephadex G-75 gel as described for cytosol [10].

### 2.5. *Immunological techniques*

Rabbits were immunized according to [19] against rat-liver Z-fraction isolated as in [10]. The immunological identification of fractions 1, 2 and 3 was performed without further purification of the antiserum according to [20].

## 3. Results and discussion

The isolation of 3 FABF from labelled rat liver cytosol was performed by a commonly used procedure in our laboratory, collecting the elution volumes of the fractions in Sephadex gel chromatography ($n$ = 52).

The radioactivity of added FA corresponded in gel chromatography to an $A_{280}$ peak and to a protein peak detected by the method in [17] for fractions 1, 2 and 3. Four different experimental methods were used to show that the 2 fractions with lower molecular weights (peaks 2, 3) were not degradation products of fraction 1 and that peak 3 was really a peptide.

First we investigated the reaction of peaks 1, 3 in column electrofocusing after they were labelled with [$^3$H]palmitic acid or [$^{14}$C]linoleic acid, respectively. After column electrofocusing of fraction 3 the $^3$H-activity was found over pH 1.3–1.7 and 8.4–9.2 ($n$ = 4) and the $^{14}$C-activity over pH 2.3–2.6 and 8.8–9.2 ($n$ = 3). Calculation of the specific radioactivity showed an unequal distribution of palmitic and linoleic acid between the alkaline and acid isoelectric points (fig.1). Whereas the distribution of

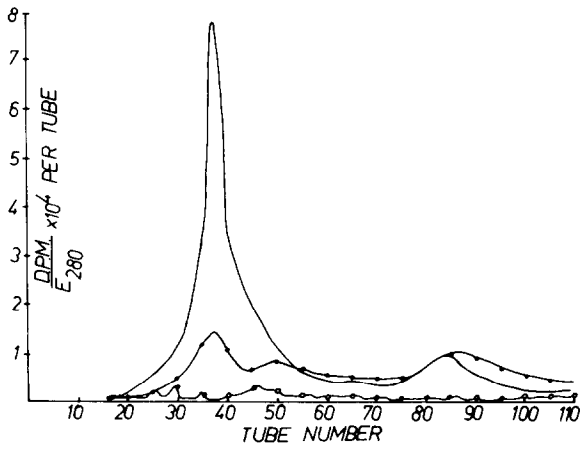


Fig.1. Specific radioactivity after column electrofocusing of fraction 3 labelled with: (———) [$^{14}$C]linoleic acid; (·—·) [$^3$H]palmitic acid; (o—o) [$^{14}$C]linoleic acid after digestion with pancreatin at 37°C for 20 h.

radioactivity in peaks 2, 3 was the same as we found in fraction 1 the radioactivity being in a peak over pH 4–5. We did not investigate the physicochemical reasons of this electrofocusing pattern but we suggest from the results that FA–protein complexes of fraction 1 were different from those in fractions 2, 3. After hydrolysis of labelled fraction 3 with pancreatin, the typical distribution in column electrofocusing was destroyed.

Second, after electrophoresis of FABF 1, 2 and 3 in SDS–polyacrylamide gel up to 18 protein fractions of peak 1 (mol. wt 30 000–100 000) and 3 protein fractions of peak 2 (main fraction mol. wt 10 000, two minor fractions with mol. wt 25 000 and 40 000, respectively) were found. The staining of peak 3 after polyacrylamide electrophoresis was possible only if fraction 3 was delipidized before electrophoresis. We suggest that lipids bound to fraction 3 prevented its staining with Coomassie blue. We found one diffuse band.

Third, we studied the carbohydrate contents of fractions 1, 2 and 3. The results are shown in table 1. The quantity of carbohydrate in relation to the amount of protein in the peak was different in the 3 fractions. The reason for this study was the fact that most plasma lipoproteins contain carbohydrates. In relation to the low molecular weight of peak 3 the carbohydrate content was very high. Therefore it was

Table 1
Carbohydrate content (nmol/mg protein) of fatty acid binding fractions of rat liver cytosol ($n$ = 3)

| | Peak 1 | Peak 2 | Peak 3 |
|---|---|---|---|
| Glucose | 3.22 ± 0.55 | 119.8 ± 42.2 | 779.9 ± 173.7 |
| Galactose | 2.78 ± 0.11 | 70.8 ± 11.6 | 41.0 ± 30.5 |
| Hexosamine | 2.23 ± 3.84 | 18.5 ± 31.7 | 23.5 ± 28.9 |
| Neuraminic acid | 4.40 ± 1.42 | 48.6 ± 15.2 | 59.5 ± 17.7 |

impossible that all monosaccharides were bound directly to one peptide chain.

Fourth, we produced a rabbit antiserum against fraction 2. As shown in fig.2 only fraction 2 and the cytosol precipitated with the antiserum. Identical results were obtained with 3 antisera from rabbits and with immunoelectrophoresis. This is definite proof of the protein heterogenity of the 3 peaks.

Summarizing our results, we suggested that no FABF of liver cytosol is part of, or degradation product of, another one. The existence of 3 FABF led us to the idea that the 3 fractions were involved in dif-

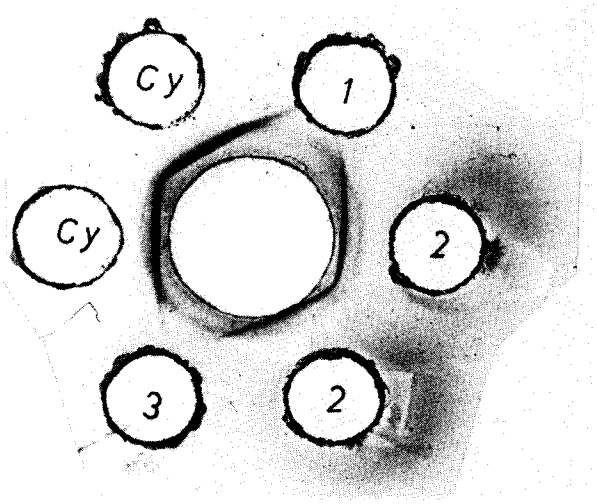


Fig.2. Immunological reaction of fatty acid binding fractions 1, 2 and 3 and liver cytosol (cy) with antiserum against fraction 2.

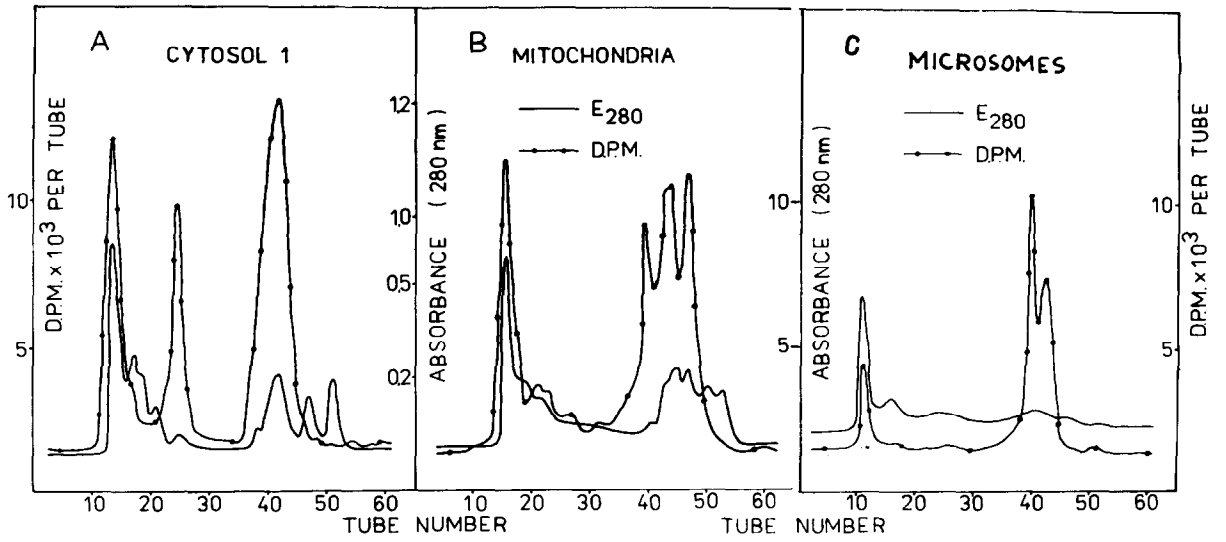


Fig.3. Sephadex gel chromatography of rat liver cytosol and 'pseudo-cytosol' from mitochondria and microsomes.

ferent metabolic pathways. But only for fraction 2 have two metabolic functions been described, it stimulated the synthesis of neutral lipid in microsomes [8] and it transferred FA for combustion into mitochondria [21]. These functions involved the interaction of the organelles with the cytosol fraction. Therefore we investigated the FA binding capacity of proteins to the organelles. We partially destroyed mitochondria or microsomes (see section 2) and the 'pseudo-cytosol' obtained was labelled and fractionated in the same manner as liver cytosol. As shown in fig.3 the distribution of radioactivity accorded with peaks 1, 3 of liver cytosol. The same results were obtained from 'pseudo-cytosol' prepared by solubilisation of the organelles with 0.5% SDS solution. From these results we suggest that some of the fatty acid binding proteins of fraction 1 originate mainly from mitochondria. The fraction 3 seems to be part of mitochondria and microsomes. The 'Z'-fraction was localized in cytosol only.

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