SPECIFIC BINDING OF SATURATED AND UNSATURATED FATTY ACIDS ON THE 'Z'-PROTEIN OF RAT LIVER CYTOSOL

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1. Introduction

The existence of a fatty acid-binding protein was first described in [1–3]. Few papers have since been published checking the binding affinities of the protein to fatty acids and the influence of the so-called 'Z'-protein on metabolic processes. Investigations concerning the function of the 'Z'-protein have shown that the triacylglycerol synthesis from dioleoyl-*sn*-glycerol was stimulated by the addition of 'Z'-protein [4]. The same stimulatory effect of 'Z'-protein was also found on the formation of monoacylglycerophosphate from *sn*-glycerol-3-phosphate [5]. In contrast, the acylation of 1-acylphosphatidylcholine and the activity of long-chain acyl-CoA-synthetase has not been affected [6]. The affinity of different fatty acids to this cytosol protein increased with the fatty acid chain length and decreased with the degree of unsaturation [7].

We believe that studies on the fatty acid-binding cytosol protein are of considerable interest for intracellular transport and metabolism of fatty acids.

This report describes our findings concerning the fatty acid pattern in the 'Z'-protein and different binding affinities of this protein to saturated and unsaturated fatty acids, respectively.

2. Methods

2.1. *Preparation of cytosol*

Male Wistar rats were killed by decapitation, the livers were quickly removed and rinsed in 0.25 M sucrose, 0.001 M EDTA (pH 7.4) at 4°C. The livers were sliced and homogenized in the same sucrose solution with a Potter-type homogenizer (1 g liver/5 ml sucrose solution). The homogenate was centrifuged at 1000 × *g* for 10 min, the supernatant then centrifuged at 20 000 × *g* for 20 min followed by centrifugation of this supernatant at 105 000 × *g* for 60 min. All operations were done at 4°C. The 105 000 × *g* supernatant, excluding the floating fat layer was stored at −20°C in ml portions for max. 2 weeks.

2.2. *Binding of radioactive labelled fatty acids to cytosol or isolated 'Z'-protein*

[^{3}H]Palmitic acid or [^{14}C]linoleic acid (Amersham) 20–100/μCi, dissolved in hexane were filled in tubes and the hexane removed by an N_2-stream at 40°C. So that the fatty acid coated the glass wall as a thin film. In the same tube 10 ml cytosol was shaken for 30 min at room temperature.

2.3. *Gel filtration on Sephadex G-75 superfine*

Sephadex G-75 column chromatography (column: 2.5 × 100 cm) was performed at 4°C with 0.15 M phosphate buffer (pH 7.4) as the elution solution with a 25 ml/h flow rate. The eluate was collected in 10 ml fractions and continously monitored at 280 nm with a Uvicord spectrophotometer (LKB-Producter AB, Sweden). For the determination of radioactivity in the eluate, 50 μl aliquots of each fraction were dried in scintillation vessels dissolved in Soluene 100 (Packard Instrument Co. Inc.) then mixed with toluole-base cocktail (4 g PPO+ 40 mg POPOP+ 10 ml ethanol/liter toluole) and counted (LKB Wallac 82 000).

2.4. *Electrofocusing of 'Z'-protein*

Electrofocusing of 'Z'-protein was carried out in the 110 ml electrofocusing column at 10°C (LKB Producter AB, Sweden). The pH-gradient was produced with Ampholine (LKB Producter AB, Sweden) pH 3–10. After 72 h at 400 V the solution in the column was collected in fractions of 1 ml and in each of the fractions the $A_{280\ nm}$, the pH, and in 50 μl aliquots, the radioactivity, was determined.

2.5. *Analysis of endogenous fatty acid pattern of cytosol fatty acid-binding proteins*

After gel filtration the fatty acid-binding proteins were extracted as in [8]. The methylesters of the fatty acids were prepared with BF_3/methanol (14%, w/v) as in [9]. All analyses were carried out in a Varian model 2100 gas chromatograph equipped with a hydrogen flame ionization detector and with a Varian model 480 integrator. The glass column measured 6 ft × 1/4 in. and contained 10% EGSS-X on Gaschrom P (100–120 mesh). The analyses were performed with programmed temperature from 150–190°C at 1°C/min. Nitrogene was used as the carrier gas with a 20 ml/min flow rate.

3. Results and discussion

A typical result of gel filtration on Sephadex G-75 of cytosol labelled with [^{3}H]palmitic acid or [^{14}C]-linoleic acid is shown in fig.1. The measurement of radioactivity in each fraction of the eluate gives three peaks of radioactivity associated to protein. We have named the peaks according to their molecular weight, 1, 2 and 3. According to [7], peak 1 represents albumin, peak 2 the 'Z'-protein, and peak 3 possibly a little fatty acid-binding peptide.

Table 1 shows the results of the gas chromatographic analysis of the endogenous fatty acid pattern of peaks 1–3. This pattern reflects the existing intracellular distribution. The data indicate a preference of 'Z'-protein for unsaturated fatty acids compared to albumin (summarized in the final line of table 1).

The problem of specific fatty acids binding also has been studied [4] with another technique. The interaction of 'Z'-protein with added specific fatty acids was measured as the percentage of bound fatty acid in relation to the added quantity of the fatty

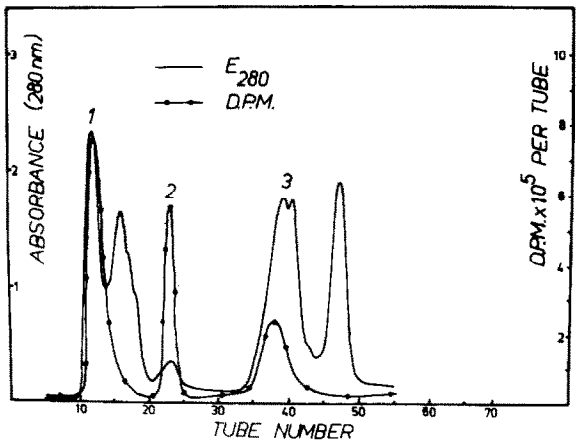

Fig.1. Gel filtration on Sephadex G-75 superfine of rat liver cytosol after incubation with [^{3}H]palmitic acid.

acid. In this way they obtained the same result: 'Z'-protein binds a higher percentage of unsaturated fatty acid (especially oleic acid) than saturated ones.

The electrofocusing of 'Z'-protein was performed with the idea that different proteins of peak 2 could

Table 1
Fatty acid distribution in fatty acid-binding proteins of rat liver cytosol

Fatty acids[a]	Peak 1	Peak 2	Peak 3
16:0	22.5 ± 3.1	21.1 ± 0.7	21.7 ± 2.0
16:1	2.5 ± 0.03	11.0 ± 1.7	14.5 ± 1.0
18:0	15.3 ± 0.1	7.8 ± 1.6	7.5 ± 1.7
18:1 (*n*-9)	14.7 ± 0.2	16.0 ± 1.1	18.3 ± 5.6
18:2 (*n*-6)	19.1 ± 1.2	15.0 ± 4.1	10.7 ± 7.6
20:0	0.4 ± 0.2	1.5 ± 0.4	1.6 ± 0.2
20:1	1.1 ± 0.01	2.1 ± 1.0	3.3 ± 1.6
20:4 (*n*-6)	11.6 ± 0.3	8.0 ± 4.7	2.7 ± 0.8
22:0	1.1 ± 0.1	1.0 ± 0.4	1.2 ± 0.4
22:5 (*n*-4)	1.7 ± 0.4	0.9 ± 0.1	1.7 ± 1.4
22:6 (*n*-3)	5.4 ± 0.3	3.5 ± 0.6	0.9 ± 0.7
24:0	0.3 ± 0.2	0.6 ± 0.1	2.1 ± 2.4
24:1	0.9 ± 0.5	1.9 ± 0.7	2.4 ± 3.1
Σ unsaturated	1.45	1.83	1.48
Σ saturated			

[a] Fatty acids of <1% were not shown

Mean value ± SD (% total fatty acid) $n = 4$

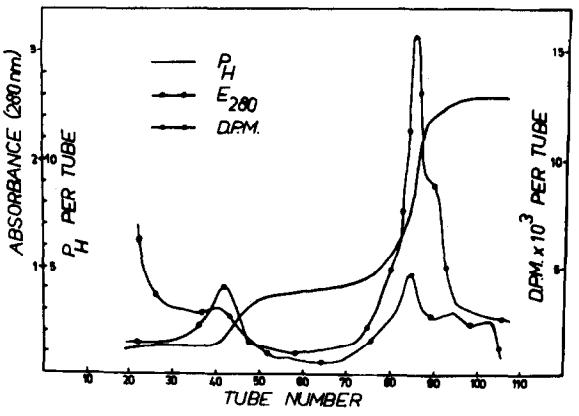

Fig.2. Electrofocusing of peak 2 ('Z'-protein) isolated by gel filtration after incubation of the cytosol with [^{3}H]palmitic acid.

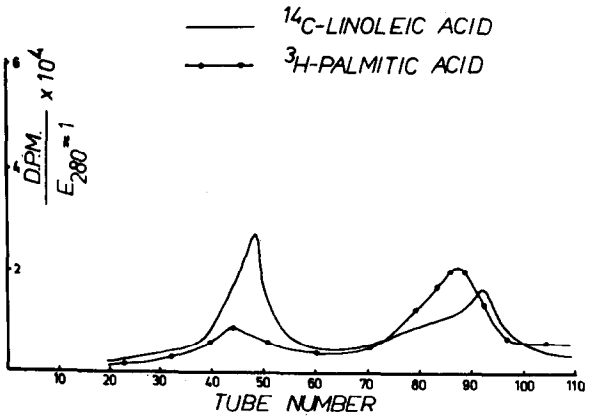

Fig.4. Relation between the radioactivity of fatty acids and protein calculated from the results of fig.2 and fig.3 expressed as specific radioactivity (dpm/E_{280} = 1).

be responsible for the binding of saturated or unsaturated fatty acids. In the electrofocusing experiments the [^{3}H]palmitic acid was found constantly in the pH ranges 1.6–1.9 and 8.8–9.2 (fig.2). The [^{14}C]-linoleic acid was localized in the pH ranges 2.2–2.6 and 8.8–9.3 (fig.3). The calculation of the specific radioactivity shows that there is an unequal distribution of palmitic and linoleic acid between the proteins with alkaline and acid isoelectric points (fig.4).

Our next experimental step was aimed at obtaining an answer to the question whether only the specific affinity of the proteins was responsible for the binding of the fatty acids, or if in vivo, for example, energy-dependent transfer reactions in the cytosol during the incubation with fatty acids are involved. In checking this problem we first isolated 'Z'-protein by gel filtration followed by an incubation of the protein obtained with [^{3}H]palmitic (fig.5) or [^{14}C]-linoleic acid (fig.6).

Both techniques, incubation of the fatty acids with cytosol or incubation with the isolated 'Z'-protein (peak 2) give similar results in the distribution of

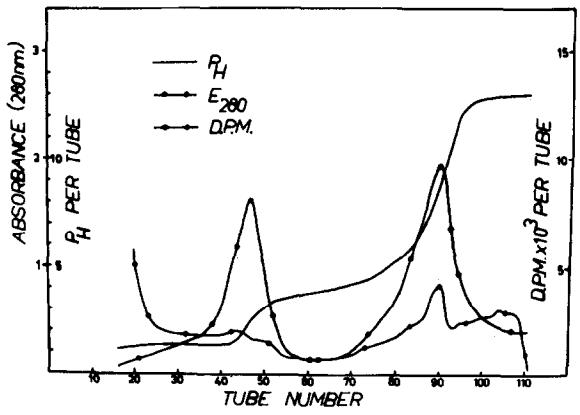

Fig.3. Electrofocusing of peak 2 ('Z'-protein) isolated by gel filtration after incubation of the cytosol with [^{14}C]linoleic acid.

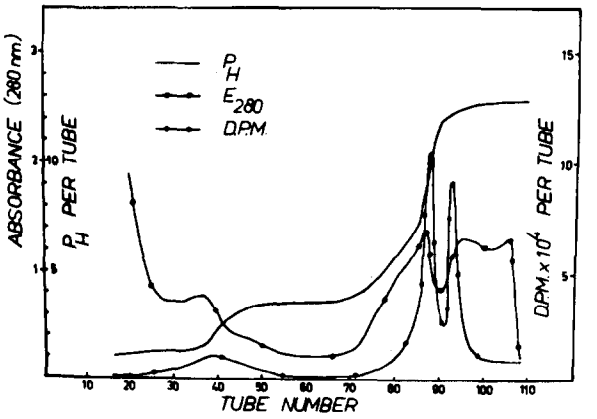

Fig.5. Electrofocusing after incubation of the gel-chromatographically isolated peak 2 with [^{3}H]palmitic acid.

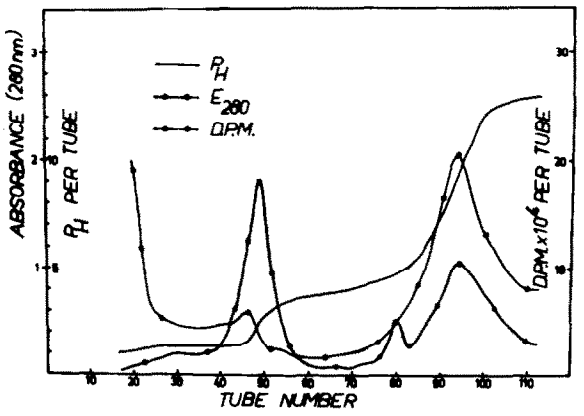

Fig.6. Electrofocusing after incubation of the gel-chromatographically isolated peak 2 with [^{14}C]linoleic acid.

proteins and in radioactivity after electrofocusing.

Our results led us to the conclusion that rat liver cytosol contains different proteins which specifically bind saturated or unsaturated fatty acids. These proteins have nearly the same molecular weight but different isoelectric points. But it is also possible that peak 2 is composed of subunits which are separated in electrofocusing experiments. Further experiments are necessary to answer this question.

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