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Expression of liver-specific genes coding for plasma proteins in protein deficiency

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Protein deficiency leads to a decreased concentration of plasma proteins, although it is not clear whether this response is caused by alterations in gene transcription or in post-transcriptional events. The aim of this study was to investigate the expression of some liver-specific genes coding for plasma proteins in rats kept on a protein-free diet for 30 days. Cloned cDNA probes for the albumin, transthyretin, retinol-binding protein and prothrombin genes were used in Northern hybridizations to total liver RNA to compare their transcript levels in protein-deficient and control animals. Liver polysomes were also isolated and fractionated from the two groups of animals to examine the possible effects of protein deficiency on translation of the mRNAs. The results indicate that the albumin and transthyretin mRNAs are present in lower amounts in protein deficiency. The distribution profile along sucrose gradients shows that all mRNAs are undergoing translation, but in protein-deficient animals a small but consistent fraction of each mRNA is also present in the non-polysomal, low molecular weight fractions.

Protein deficiency; Plasma protein; Gene expression; (Liver)

1. INTRODUCTION

During protein-energy malnutrition, changes in liver protein synthesis have been reported to occur, both in man [1,2] and in experimental animals [3,4]. Although the concentration of most plasma proteins is greatly diminished in protein deficiency, studies undertaken to uncover the mechanisms of the decrease in the level of individual proteins have yielded conflicting results, due to the differences in the severity of the deficiency as well as in the methods used to measure the amount of protein synthesized. It is yet to be elucidated whether the final response is caused by alterations in gene expression, post-translational modifications during secretion, stability of the proteins or by a combination of more than one of these factors [5].

Many studies have demonstrated a decreased RNA content, secondary, according to some authors, to an increase in nuclease activity, possibly due to lysosomal activation or to depression of the ribonuclease inhibitor activity [6,7]. The amount of specific mRNAs in the cell, in altered nutritional conditions, has been estimated in the past years in cell-free protein synthesizing systems followed by immunoprecipitation of the proteins with specific antibodies [8,9]. However, the efficiency of translation of an mRNA in vitro does not necessarily correspond to the efficiency of its translation in vivo, and this method does not distinguish between transcriptional and post-transcriptional regulatory steps.

The availability of cDNA clones has made it possible to measure the levels of specific mRNA species by means of hybridization techniques [10]. In the present paper we have examined the expression in the liver of some genes coding for plasma proteins, following the induction of a severe protein-energy deficiency. Specific cDNA clones for the plasma proteins albumin, transthyretin (TTR), prothrombin and retinol-binding protein (RBP), as well as those coding for the cell proteins L-ferritin, β-actin and cellular RBP (CRBP), have been used as probes to investigate whether protein deficiency affects the expression of the genes coding for secretory proteins, and if the possible regulatory steps are common to all the proteins within this class.

2. MATERIALS AND METHODS

Male Sprague-Dawley rats (150 ± 10 g) were fed a protein-free diet or a 20% casein diet ad libitum for 30 days. At the end of the experimental period, the animals were sacrificed, the liver was dissected out and either frozen immediately in liquid nitrogen, or perfused with 0.9% NaCl containing 150 μg/ml cycloheximide and immediately processed for the preparation of polysomes.

Total polysomes were prepared from fresh livers by a modification of the method described by Aziz and Munro [11]. Post-mitochondrial

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Abbreviations: CRBP, cellular retinol-binding protein; RBP, retinol-binding protein; TTR, transthyretin (prealbumin); PD, protein deficient

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supernatants (PMS, 15 min at 23500 × g) were adjusted to 0.5% Triton X-100, 0.5% Na deoxycholate, 1% Tween 40, layered over a 10–50% linear sucrose gradient and centrifuged at 26000 rpm for 4 h in a Beckman SW 27 rotor. 15–16 fractions of 2 ml each were collected from each gradient for RNA extraction.

Total RNA was extracted from frozen pulverized liver by homogenization in 5 M guanidine thiocyanate followed by centrifugation over a 5.7 M cesium chloride cushion as described by Chirgwin et al. [12]. Polysomal fractions collected from the gradient were treated with proteinase K (200 μg/ml, 30 min at 37°C), phenol-extracted, and the RNA was ethanol-precipitated and stored at −70°C until further use.

Total RNA or polysomal RNA (10–20 μg/lane) were fractionated on 1.5% agarose gels in 6% formaldehyde, blotted onto nylon membrane (Hybond N, Amity pg), and hybridized to cDNA probes, radioactively labeled with ^{32}P by nick-translation [13].

Slot-blots were obtained by immobilization of RNA onto nitrocellulose filters using the Micro-sample filtration Manifold produced by Schleicher and Schuell, following the protocol supplied by the manufacturer. Densitometric scanning of the autoradiographies was performed using an LKB Ultroscan XL Laser Densitometer.

Hybridizations were carried out at 42°C for 16 h in 50% deionized formamide, 5 × SSPE (1 × SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, pH 7.7, 1 mM EDTA), 0.5% SDS, 5 × Denhardt's (1 × Denhardt's is 0.02% Ficoll, 0.02% BSA, 0.02% polyvinylpyrrolidone), 10% dextran sulphate. The hybridized filters were washed twice in 2 × SSC (1 × SSC is 0.15 M NaCl, 15 mM Na citrate, pH 7.0), 0.1% SDS, followed by two washes in 1 × SSC, 0.1% SDS. All washes were at 60°C for 20 min.

The cDNA clones used in this study were: human RBP [14], murine TTR [15], human albumin [16], human prothrombin [16], human CRBP [17], rat L-ferritin [18], rat β-actin.

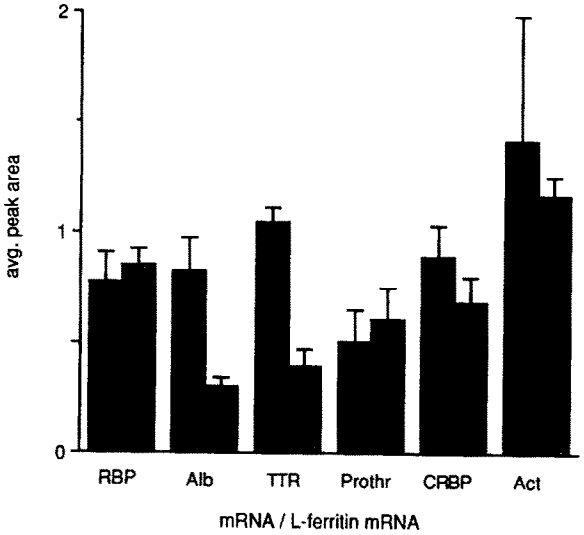

Fig.1. Comparison of the relative amounts of specific mRNAs in the liver of protein-deficient (hatched bars) and control rats (solid bars). The values obtained by densitometric scanning of the autoradiographies are normalized to the amount of L-ferritin mRNA in each sample to correct for individual variations. Each value, expressed in arbitrary units, is the average of at least 3 independent determinations ± SE. Since the time of exposure necessary to reach the linear range for densitometric scanning is different for each filter, the values in the figure do not reflect the relative abundance of the various mRNAs in liver cells.

3. RESULTS AND DISCUSSION

3.1. *Plasma protein mRNA transcription in the liver of protein-deficient rats*

The circulating level of most plasma proteins is known to decrease dramatically in protein deficiency [1]. Therefore, we have compared the level of specific plasma protein mRNAs in the liver of protein-deficient and control rats. Under the same experimental conditions that we have used in this study, the level of total plasma proteins and that of circulating albumin in the protein-deficient animals have been reported to decrease to about 65% of their respective control values [19].

In order to determine quantitatively the possible variations in the level of several specific mRNAs in protein deficiency, we initially performed slot blot hybridizations. 5–10 μg aliquots of total RNA extracted from the liver of at least 5 animals from each group were spotted onto multiple strips of nitrocellulose, which were then separately hybridized to ^{32}P-labeled cDNAs. The resulting autoradiographies were analyzed by laser densitometry, and the values obtained, normalized to the level of L-ferritin mRNA in each slot, are reported in fig.1. As shown in fig.1, the normalized levels of the albumin and TTR mRNAs decrease about 2.5-fold in the protein-deficient animals with respect to the controls. The steady-state level of the other mRNAs that we measured does not seem to vary in the two groups of animals. Only the average value of the actin mRNA displays a very high variability, due to the great deal of individual variation that we have observed for this mRNA, which in the PD group is counterbalanced by a larger number of samples represented by the mean value.

We further analyzed the albumin and TTR steady-state mRNA levels by Northern hybridization of total liver RNA from the two groups of animals to the cloned cDNA probes, as shown in fig.2. Multiple lanes containing total RNA extracted from the liver of different animals were loaded on the gel, to correct for the individual variations that are often observed in nutritionally deprived animals. In all PD animals tested, the albumin and TTR mRNA levels were decreased with respect to the levels observed in their corresponding controls. Hybridization of the same filters with cDNA probes encoding β-actin, L-ferritin and RBP serves as internal control for both intracellular and plasma proteins whose mRNA level is not affected by protein deficiency, in agreement with the data concerning the level of such proteins in this nutritional deprivation [20].

3.2. *Distribution profile of the mRNAs on fractionated liver polysomes*

Of the plasma proteins whose circulating levels are known to decrease in protein deficiency, the RBP-TTR complex is considered to be one of the most sensitive indicators for the assessment of malnutrition [21]. Our data demonstrate that transcription of the TTR gene is dramatically affected in the liver of protein-deficient

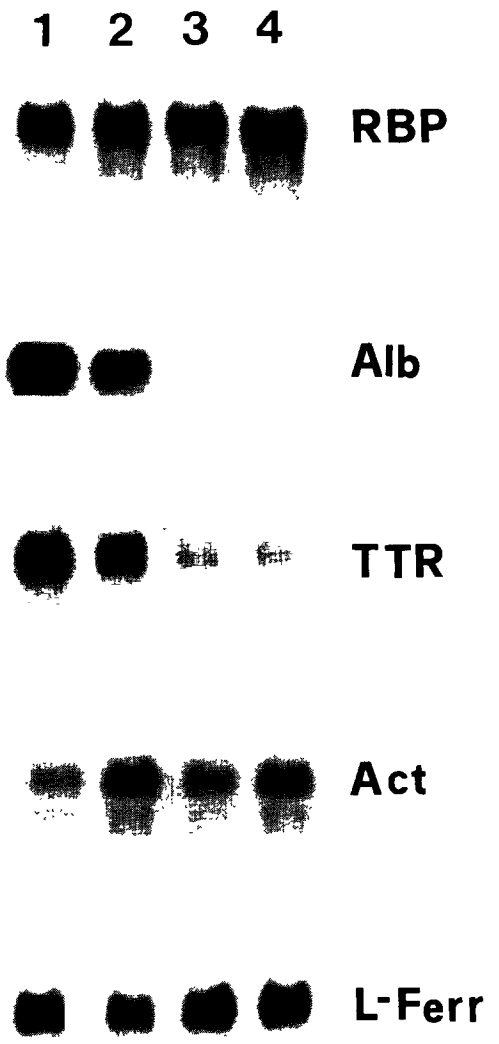

Fig.2. Northern hybridizations of total liver mRNA from control (lanes 1 and 2) and protein deficient (lanes 3 and 4) rats to the ^{32}P-labeled cDNAs indicated. Each lane represents one animal and contains 10 μg of total liver RNA. Since the time of exposure is different for each filter, the values in the figure do not reflect the relative abundance of the various mRNAs in liver cells.

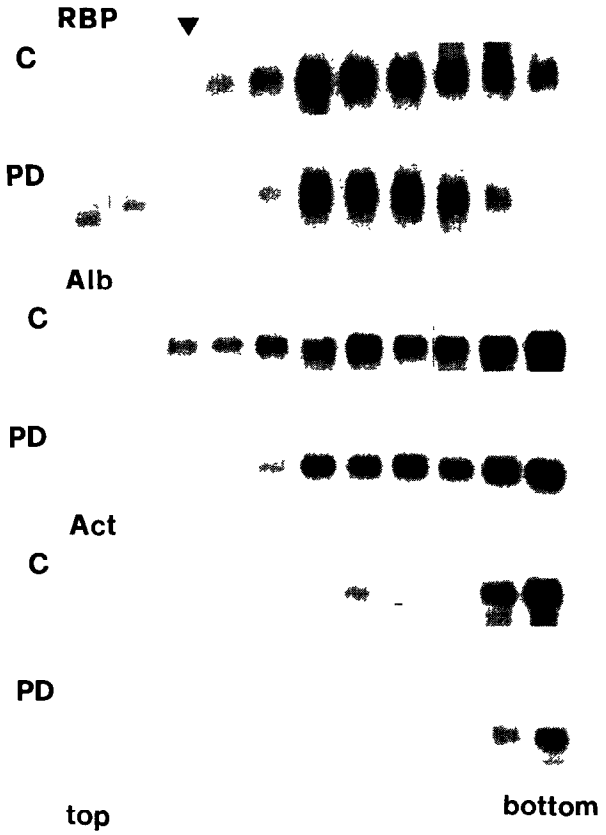

Fig.3. Distribution of the RBP, albumin and actin mRNAs in the fractionated liver polysomes from control (C) and protein-deficient (PD) rats. 10 μg of the RNA extracted from each fraction were loaded in each lane. The arrow indicates the position of 80 S monomeric ribosomes in the gradient, as determined by ethidium bromide fluorescence staining of the agarose gels and optical density measurements at 260 nm wavelength.

animals, although we did not observe any significant variation in the level of RBP mRNA. Therefore, we extended our analysis to the efficiency of translation of this mRNA. We fractionated liver polysomes by sedimentation on linear sucrose gradients, extracted RNA from each fraction and analyzed the distribution profile of each specific mRNA onto fractionated polysomes by Northern hybridization with the corresponding cDNA probe. A comparative analysis of the translational efficiencies of the RBP, albumin and actin mRNAs in the liver of the two groups of animals is shown in fig.3. In both PD and control gradients (C) the mRNAs are present in the polysomal fractions according to their size. Only in the liver of PD animals a small but consistent fraction of each mRNA is also present in the lighter fractions, which contain non-polysomal material that is not being translated. The ethidium bromide fluorescent staining of the gels confirms the presence of comparable amounts of RNA in all fractions from all gradients. This seems to be a

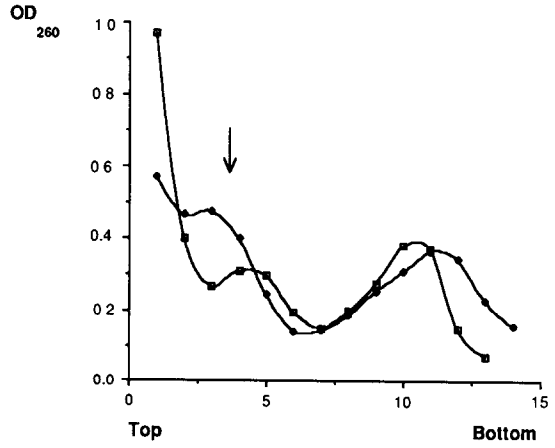

Fig.4. Optical density profile at 260 nm wavelength of gradient fractions containing liver polysomes from control (open squares) and protein-deficient (solid squares) rats.

general effect, common to all mRNAs that we have tested, coding for both plasma and intracellular proteins. Moreover, the optical density measurements of the fractions from these gradients evidence a large increase in the accumulation of free monomer ribosomes in the liver of PD rats (fig.4), in agreement with previously reported data (reviewed in [22]). Taken together, these data suggest that the availability of some factor involved in the initiation of translation is reduced in protein deficiency. Such mechanism has been previously reported to occur in nutritionally deprived animals and in tissue culture cells [23], although it does not seem to play a major role in the regulation of protein synthesis in response to amino acid starvation [24].

The data reported in this paper demonstrate a clear correlation between the reduced hepatic level of albumin and TTR mRNAs and the decreased protein level known to occur in protein deficiency, even though our approach cannot identify the specific signal(s) involved in this regulation. Transcription of the RBP gene, however, is not affected. We believe that the reduction in hepatic retinyl palmitate hydrolase activity that occurs in protein deficiency [25], can mimic a condition of retinol deficiency and therefore result in a secretory block of the RBP.

We conclude from our analysis that nutritional regulation of plasma proteins can occur at different levels, and that the genes encoding this class of proteins are not regulated by a common mechanism.

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