Volume 210, number 2, 173–176 FEB 04377 January 1987

Retention of actin synthesis in liver under conditions that inhibit synthesis of almost all other proteins

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Received 15 October 1986

As briefly reported [(1986) Fed. Proc. 45, 1771, Abstr. 1690], rats fed a protein-free diet for a few days often show a marked inhibition of protein synthesis in liver cytosol. However the synthesis of a protein of molecular mass ~42 kDa is fully retained. We show here on the basis of its molecular mass, number of bands on isoelectric focusing, isoelectric point and immunological reactivity that this protein is actin and also that actin mRNA is not degraded by micrococcal nuclease under conditions which degrade the bulk of other mRNAs.

Actin; Protein synthesis; (Liver)

1. INTRODUCTION

Protein-free diet dramatically reduced (i.e. by 90%) protein synthesis in liver post-mitochondrial supernatants [1] at the level of initiation. However, there is a protein of ~42 kDa whose synthesis is maintained. This protein shows two bands on isoelectric focusing with a p*I* around 5.4 as is the case for liver actin. Moreover, it binds specifically to anti-actin antibody. On these bases it is concluded that this protein is actin. It is also shown that when the bulk of protein synthesis is inhibited by degrading mRNA by treatment with micrococcal nuclease the synthesis of actin remains unaffected.

2. MATERIALS AND METHODS

2.1. *Animals and diets*

Male rats of the Wistar strain weighing about 180 g were used. Protein-free diet (protein depletion diet U.S.P. XV) was from ICN Nutritional Biochemicals (Cleveland, USA). Standard diet (20% protein) was Sandermus, from Sanders (Spain). Rats were fed the protein-free diet for 3 days.

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2.2. *Cell-free protein synthesis*

Liver protein synthesis was assayed essentially as in [2] and brain protein synthesis as in [3]. The standard synthesis mixture (15 μl) contained 80 mM KCl, 0.3 mM $MgCl_2$, 10 mM creatine phosphate, 1 μg creatine kinase, 5 μl rat liver postmitochondrial supernatant, 4 μCi [^{35}S]methionine (1200 Ci/mmol, 11 mCi/ml) and a mixture of the remaining 19 amino acids (50 μM each). Samples were incubated for 30 min at 30°C and trichloroacetic acid-precipitable radioactivity was counted as in [2].

2.3. *Treatment with micrococcal nuclease of liver and brain extracts*

The treatment is based on [4]. Reaction mixtures contained in a final volume of 200 μl, 25 mM Tris-HCl, 2.5 mM $MgCl_2$, 12.5 mM KCl, 12.5 mM NH_4Cl, 2.5 mM 2-mercaptoethanol, 0.125 M sucrose, 0.5 mM $CaCl_2$, 1 mM phosphocreatine, 10 μg creatine kinase, 10 μM ATP, 2 μM GTP, pH 7.6, 90 μl of the cell-free system and 2, 8 or 16 U

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00145793/87/$3.50

micrococcal nuclease. After 10 min at 20°C, 10 μl of 0.25 M EGTA were added to inhibit the nuclease.

3. RESULTS AND DISCUSSION

As indicated in section 1, while testing the effect on liver protein synthesis of feeding rats a protein-free diet we found this to be often dramatically reduced (i.e. by 90%). The products of synthesis were subjected to SDS-polyacrylamide gel electrophoresis and fluorography. As shown in fig.1, a post-mitochondrial supernatant from livers of rats fed standard diet synthesizes many proteins, however the pattern of proteins synthesized by post-mitochondrial supernatants from livers of rats fed a protein-free diet is remarkably different. Only a few bands are detectable and there is a protein of ~42 kDa which represents about 40% of incorporated radioactivity as determined by densitometric scanning. As shown in fig.1, this protein in the cytosol of livers from rats on a standard diet represents only about 6% of the total protein synthesized, while for rats on a protein-free diet, with 10% of total protein synthesis, it represents about 40%, indicating that translation for this protein is preferentially maintained in rats fed a protein-free diet.

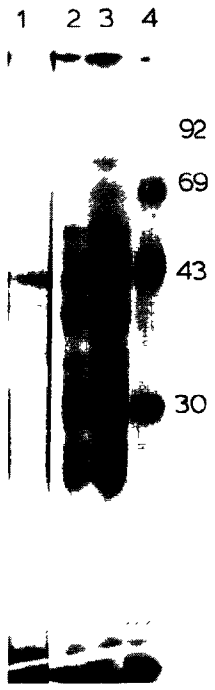

Fig.1. Maintenance of synthesis of a protein of 42 kDa in post-mitochondrial supernatants from livers of rats fed protein-free diet. Cell-free protein synthesis was carried out as described in section 2. Samples were subjected to SDS-polyacrylamide gel electrophoresis using 12% gels. The gel was dried and treated for fluorography [10]. Lanes: 1, protein-free diet; 2,3, standard diet; 4, molecular mass markers (in kDa). Because of the different amounts of radioactivity present lane 1 was exposed for 2 weeks and lanes 2–4 for 2 days.

On the basis of its molecular mass, it seemed possible that this protein was either creatine kinase or actin. In vertebrates creatine kinase occurs as three isoenzymes with p*I* around 6.1 [5]. Liver contains two isoactins (β and γ) with p*I* 5.42 and 5.44 [6,7]. When the 42 kDa protein was subjected to isoelectric focusing two bands were detected with p*I* around 5.4 (fig.2) as is the case for actin.

The binding of the protein to anti-actin antibody was investigated using an assay in which the specific anti-actin antibody or control serum was attached to immunotitration plates. The synthesis mixture containing the labeled protein was added, incubated and the radioactivity bound to the antibody counted. As shown in table 1, control serum does not bind radioactivity while anti-actin antibody binds up to 41% of the radioactivity added, in excellent agreement with the 40% radioactivity corresponding to the protein of ~42 kDa. These results clearly indicate that the protein for which synthesis is preserved is actin.

The protein-free diet inhibits liver protein synthesis at the level of initiation (to be published).

It seems that preferential preservation of actin synthesis could reflect one or more of the following: (i) elongation of previously initiated chains; (ii) actin initiation being able to proceed at lower levels of initiation factors; (iii) initiation proceeding through a mechanism different from that of most proteins; in this regard it has been recently suggested that ACG can function as a low-efficiency initiation codon in a wheat germ cell-free protein-synthesizing system [8]. It is possible that, in addition to the better known mechanism of initiation of protein synthesis, the eukaryotic cells possess other initiation mechanisms with somewhat different requirements. For example, it

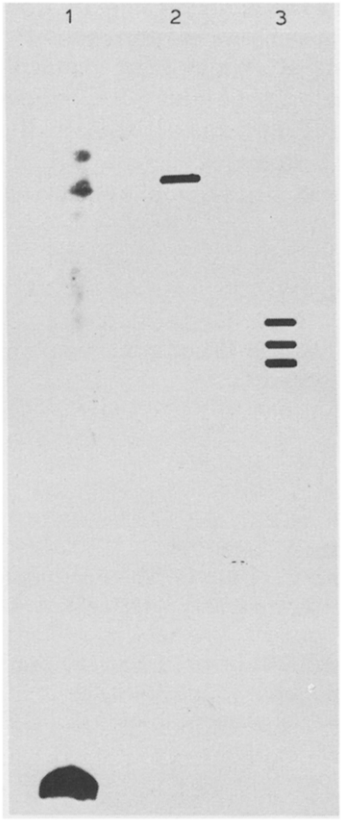

Fig.2. Determination of the isoelectric point. Isoelectric focusing was carried out as described in [11] using Bio-Lyte 5/7. Solutions containing 1 mg/ml of insulin (pI = 5.32) and creatine kinase (from Sigma) (pI ≃ 6.1) were used as markers. 5 μl of the standards and of the translated cell-free system were applied. After isoelectric focusing the gel was fixed, the standards stained with Coomassie blue and their positions recorded. The gel was treated for fluorography [10], dried and X-Omat XS5 films were exposed for 6 days. Lanes: 1, product synthesized by liver post-mitochondrial supernatants from rat fed a protein-free diet; 2, position of insulin (pI = 5.32); 3, position of creatine kinase isoenzymes (pI ≃ 6.1).

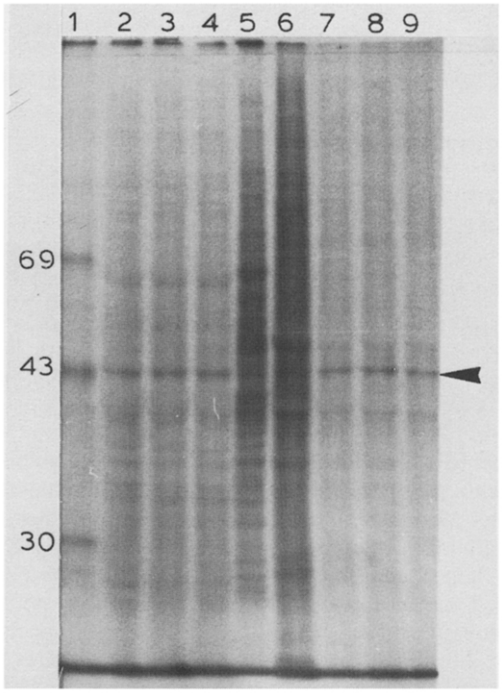

Fig.3. Actin mRNA is protected against micrococcal nuclease in both liver and brain post-mitochondrial supernatants. Liver and brain post-mitochondrial supernatants were treated with different amounts of micrococcal nuclease as described in section 2. Protein synthesis was carried out for 30 min at 30°C. 3 μl were taken to count trichloroacetic acid-precipitable radioactivity and to the remaining sample were added 30 μl of a buffer containing 12.5% glycerol, 1.25% SDS, 1.25% 2-mercaptoethanol, 0.125% bromophenol blue, 62 mM Tris-HCl, pH 6.8. Samples were boiled for 5 min and subjected to SDS-polyacrylamide electrophoresis using 12% gel. The gel was dried and treated for fluorography [10]. Lanes: 1, molecular mass standards; 2–4, liver post-mitochondrial supernatant treated with 2, 8 and 16 U micrococcal nuclease; 5, untreated liver post-mitochondrial supernatant; 6, untreated brain post-mitochondrial supernatant; 7–9, brain post-mitochondrial supernatant treated with 2, 8 and 16 U micrococcal nuclease. Arrowhead indicates the position of actin.

has been proposed that there is a ribosome component needed for translation of normal messages but not for translation of heat-shock messages [9].

We also tested the effect of degrading mRNA by treatment with micrococcal nuclease on protein synthesis. After nuclease treatment there was always a low, ~10%, residual protein-synthesizing activity. As shown in fig.3, a large proportion of this residual synthesis corresponds to actin, indicating that in both liver and brain cell-free systems actin mRNA is well protected against nuclease action, even when very high amounts of nuclease were used. A possibility is that there are actin mRNA molecules with attached ribosomes

Table 1

Binding to anti-actin antiserum of protein synthesized in liver post-mitochondrial supernatants from rats fed a protein-free diet[a]

Total protein added (dpm)	Corresponding to the 42 kDa band[b] (dpm)	Protein bound to			
		Control serum (dpm)	(%)[c]	Anti-actin anti-serum (dpm)	(%)[c]
54950	21980	61	0.25	580	3
3760	1500	0	0	537	36
580	232	0	0	238	102

[a] 50 μl of a 1:10 dilution of anti-actin antiserum (Miles Scientific) or of control serum were bound to immunotitration plates (96F Nunc immunoplates, Inter Med, Denmark) which were then saturated with ovalbumin and washed 3 times with PBS (10 mM sodium phosphate, pH 7.2, containing 8.5% NaCl). 50 μl of different dilutions of the protein synthesis incubations (see section 2) were added and incubated for 2 h at 22°C in a moist chamber. The plates were washed 3 times with PBS containing 20 mM L-methionine. To dissociate the antigen-antibody complex, 100 μl of 0.2 N NaOH were added to each well and incubated at 37°C for 60 min. Samples were removed and trichloroacetic acid-precipitable radioactivity bound to the anti-actin antibody or to control serum was counted

[b] Calculated from the percentage of total synthesis corresponding to the band of 42 kDa determined by densitometric scanning

[c] The percentage has been calculated on the basis of the radioactivity on the 42 kDa band

whose translation is repressed but which can be switched on rapidly when needed. This could explain why actin synthesis can proceed when initiation is inhibited. The preferential preservation of actin synthesis shown here is of interest per se and also because it indicates that actin is essential for maintaining cell function.

ACKNOWLEDGEMENTS

We thank R. Wallace for excellent assistance with the major part of this work. Supported in part by the FISS, and the IIC-KUMC International Molecular Cytology Program. V.F. is a 'Severo Ochoa' fellow of the Fundación Ferrer.

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