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IDENTICAL PROPERTIES OF AN mRNA-BOUND PROTEIN AND A CYTOSOL PROTEIN WITH HIGH AFFINITY FOR POLYADENYLATE

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SUMMARY: A rat liver soluble protein which binds very tightly to polyadenylate in vitro, and a specific mRNA-bound protein from free polyribosomes, have been isolated by techniques of affinity chromatography. The two protein preparations were compared on the basis of criteria such as stability and specificity of the binding, molecular weight, which was 78,000 in both cases, and amino acid composition. We conclude that the two proteins are identical or at least highly related species and that the cytosol component may be a precursor of the polyribosome-associated protein.

Polyribosomal mRNA from eukaryotic cells is associated with two predominant protein species of molecular weights close to 52,000 (P 52) and 78,000 (P 78) daltons (1-3). Polyribosomal P 78 forms a specific and very tight complex with the poly(A) segment at the 3' end of the mRNA (4). There are also reports in the literature indicating that in addition to the poly(A)-associated form protein P 78 exists in a free soluble state in the pool of RNA-binding factors in cell nuclei and cytoplasm (5,6). Such identification of cytosol P 78 and experiments from our laboratory identifying cytosol P 78 in Ehrlich carcinoma ascites cells (7) must be considered, however, as preliminary. We have now extended our previous work and compared preparations of cytosol P 78 and polyribosomal P 78, both isolated from a rat liver homogenate. Evidence is presented that the two samples have several structural properties in common and it appears likely that they are identical proteins.

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MATERIALS AND METHODS: [^{3}H]-adenosine (40 Ci/mmol), [^{3}H]-polyadenylate, and [^{32}P]-orthophosphate were from New England Nuclear, Dreieich, Deutschland. RNAses A (XII-A), T_1 and T_2 were products of Sigma, München. Cellulose-oligo(dT), T3, was obtained from Collaborative Research, Waltham, Mass. .

Male SD rats weighing 200 g were each injected with 100 µCi [^{3}H]-adenosine 60 min before sacrifice. The livers were removed and homogenized with 0.02 M Tris-HCl, pH 7.6, 0.05 M NaCl, 5 mM $MgCl_2$, 5 mM ß-mercaptoethanol and 0.25 M sucrose (medium A) in a Dounce homogenizer. To prepare free polyribosomes the mitochondrial supernatant of the homogenate was layered on discontinuous gradients of 0.5 M (1 ml), 1.5 M (1 ml) and 2.0 M (3 ml) sucrose in 0.02 M Tris-HCl, pH 7.6, 0.5 M NaCl and 5 mM $MgCl_2$ and centrifuged in a Spinco 50 Ti rotor at 50,000 rpm for 5 h. The polyribosome pellets were rinsed twice with medium A.

The cytosol fraction was prepared by centrifugation of the mitochondrial supernatant at 50,000 rpm for 3 h (8). In this experiment the animals were previously starved for about 60 h and livers were perfused with a solution of 0.32 M sucrose, 3 mM $MgCl_2$ in situ. The cytosol was dialysed overnight against the chromatography buffer (0.03 M Tris-HCl, pH 8.4, 1 mM EDTA, 0.1 mM DTE, 0.05 M NaCl and 10% glycerol). Cytosol P 78 was isolated by affinity chromatography on Sepharose-poly(A) essentially as described in our previous report on the purification of P 78 from Ehrlich carcinoma ascites cytoplasm (7). Binding of proteins to the polynucleotide was accomplished in a batch procedure at ice temperature. The gel was then washed successively in a column with 0.2, 0.6, 2.0 M NaCl and 6.0 M urea, each in chromatography buffer, and finally eluted for P 78 with 50% formamide, 0.5 M NaCl in the same buffer. These experiments were performed with a gel prepared from CNBr-activated Sepharose (Pharmacia, Freiburg) and potassium polyadenylate (Boehringer, Mannheim) in our laboratory (8). Commercial preparations of matrix-bound polyadenylate were found to be less suitable.

A fraction of polyribosomal P 78 was prepared from free polyribosomes according to Lindberg and Sundquist (9) as modified by Irwin et al. (10). The material eluted with 50% formamide, 0.25 M NaCl from a column of cellulose-oligo(dT) was collected and saved for analysis. Poly(A) segments were isolated from the formamide eluate in an experiment in which polyribosomes were labeled with [^{32}P]-orthophosphate for 4 h in vivo (11-15).

The isolated proteins were analysed by SDS gel electrophoresis (16) on a 7.5% acrylamide slab gel in the E-C Vertical Gel equipment (E-C Apparatus Co., Philadelphia, Pa.). Techniques for the determination of amino acid composition were those that have been described in a previous report (17). The preparation of P 78 from free polyribosomes was incubated with RNAses T_1 and T_2 at 37° and reprecipitated prior to acid hydrolysis.

RESULTS: A fraction of cytosol P 78 was prepared from liver as a nearly pure component following the procedure that we have described previously (7). Fig. 1 and 2A show that the protein in

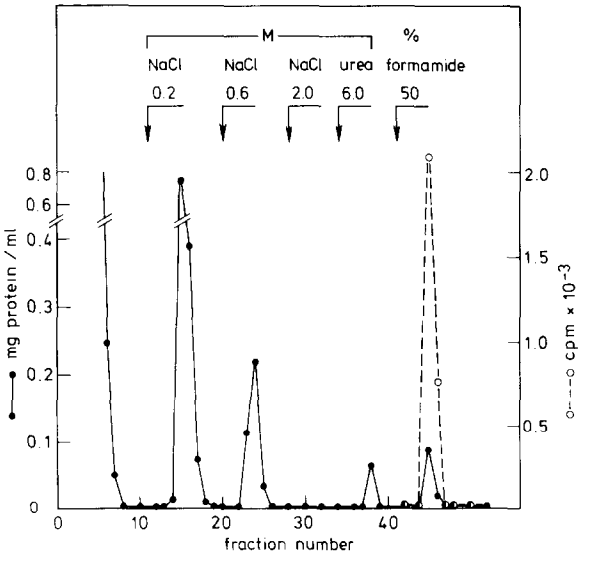

Figure 1. Affinity chromatography of cytosol proteins on Sepharose-polyadenylate. Cytosol prepared from 5 rat livers (570 mg protein) was applied to a 7 ml column and fractions of 1.5 ml were collected in a stepwise elution as indicated. o----o , polynucleotide-binding activity; 5 µl of the sample was mixed with 5 nCi of [^{3}H]-polyadenylate in 1 ml chromatography buffer and the amount of protein-bound radioactivity determined (8).

the formamide eluate interacted very strongly with Sepharose-polyadenylate during chromatography; it is thus the component with the highest affinity for poly(A) of all the cytosol proteins under the given conditions of chromatography. Fig. 1 also shows that the formamide-eluted protein retained its high affinity for polyadenylate. Interaction of the protein with certain other polynucleotides could be observed but the binding was less effective in these cases. The material obtained in the 0.2 and 0.6 M NaCl washes was also active in the polynucleotide-binding assay; such profiles of binding activity have been reported in a previous communication (8). In control experiments in which the cytosol was prepared from livers labeled with [^{3}H]-adenosine or [^{32}P]-orthophosphate for 4 h only traces of radioactivity were present

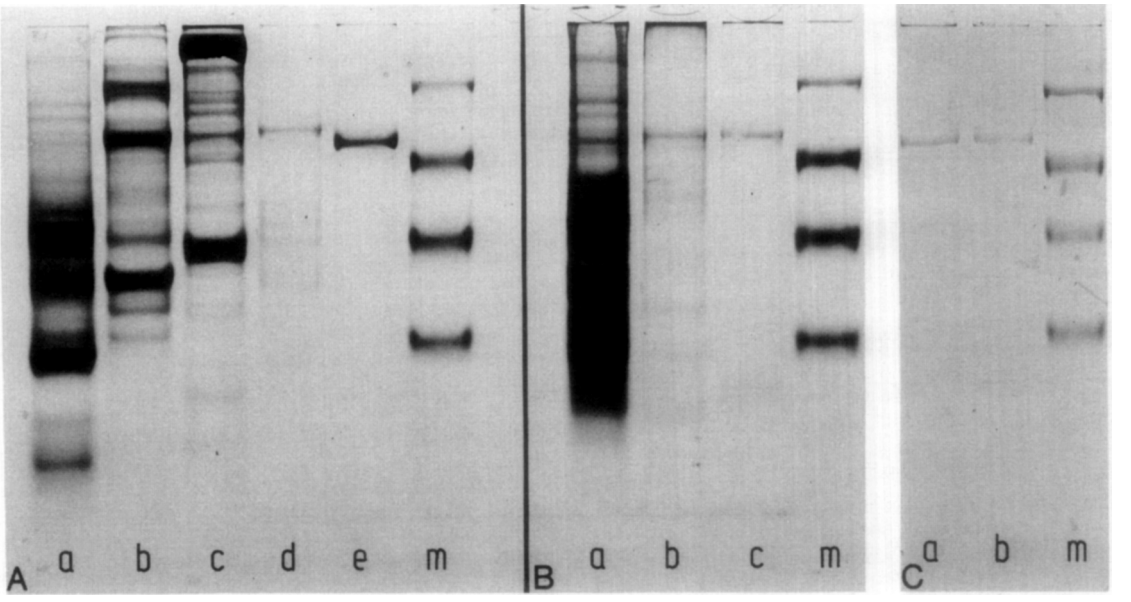

Figure 2. SDS polyacrylamide gel electrophoresis of isolated proteins. (A), analysis of cytosol proteins fractionated on Sepharose-polyadenylate: a, material not bound to the gel; b, c, d, proteins in wash fractions with o.2, o.6 M NaCl and 6.o M urea, respectively; e, protein eluted with 5o% formamide, o.5 M NaCl; m, mixture of molecular weight markers consisting of chymotrypsinogen (25,ooo), ovalbumin (43,ooo), bovine serum albumin (68,ooo) and ß-galactosidase (13o,ooo). (B), analysis of polyribosomal proteins fractionated on cellulose-oligo(dT): a, unbound material; b, c, protein eluted with 25% and 5o% formamide, respectively. (C), comparison of P 78 isolated from free polyribosomes (a) and cytosol (b).

in the formamide eluate and we therefore conclude that the cytosol P 78 was binding directly to the poly(A).

A fraction of P 78 which is bound to mRNA, polyribosomal P 78, was isolated from rat liver free polyribosomes by affinity chromatography on cellulose-oligo(dT). The material eluted with 5o% formamide from the column contained radioactively labeled RNA and one main polypeptide (Fig. 2B). Obviously, this protein became bound to the gel via the RNA in the sample since it did not bind to Sepharose-poly(A) under identical conditions of chromatography. Additional evidence that the polyribosomal P 78

existed as a poly(A)-protein complex was obtained in an experiment in which radioactively labeled segments of polyadenylate with a characteristic size (15) were identified in the formamide-eluted fractions (diagram not shown).

The yield of cytosol P 78, as determined in the 50% formamide eluate, was 30-50 μg per liver. The amount of polyribosomal P 78 recovered in the 25% and 50% formamide eluates after fractionation of free polyribosomes on the oligo(dT) column was usually in the range of 60-80 μg per liver.

Fig. 2C shows that samples of polyribosomal P 78 and cytosol P 78 both migrated as a single band when analysed by SDS electrophoresis in a polyacrylamide slab gel. The molecular weight of this component was identical in both cases and was calculated to be 78,000 ± 2,000.

The amino acid composition of the P 78 proteins is given in Table 1. There is a great similarity between the analyses and in addition it can be seen that both preparations contain relatively large amounts of aspartic plus glutamic acid (22.8% for the polyribosomal and 23.0% for the cytosol protein). The ratio of acidic to basic amino acids was 1.5 and the proteins could therefore be considered as being acidic. It is not known, however, whether and to what extent dicarboxylic amino acids were present in the proteins as asparagine and glutamine. The analysis (see Table 1, column 3) of a poly(A)-binding protein isolated from Ehrlich carcinoma ascites cell cytosol (7), and corresponding to cytosol P 78 from liver, is almost identical with the data presented here.

DISCUSSION: Our results show that polyribosomal P 78 and cytosol P 78 have a number of similar or identical properties: (i) Cytosol P 78 binds very strongly to the Sepharose-poly(A) used in its isolation. A comparable tight binding of protein to

Table 1. Amino acid composition of protein P 78.
Compositions are expressed in terms of mol per loo mol of recovered amino acids.

	P 78 isolated from		
	rat liver		Ehrlich carcinoma ascites
Amino acid	polyribosomes	cytosol	cytosol (7)
Lys	7.4	7.1	6.3
His	2.2	2.o	2.2
Arg	4.9	5.6	5.5
Asp	9.4	lo.4	9.6
Thr	5.o	5.3	5.3
Ser	7.6	7.4	7.5
Glu	13.4	12.6	14.4
Pro	5.9	6.3	6.7
Gly	11.2	9.1	lo.7
Ala	8.1	9.o	8.9
Val	5.7	6.2	5.1
Met	2.3	1.1	1.9
Ile	3.2	4.1	3.2
Leu	7.2	8.3	7.1
Tyr	2.7	1.9	3.1
Phe	3.8	4.o	2.4

the poly(A) segment of hnRNA or mRNA has been reported by several authors (13, 18, 19). (ii) The molecular weight of 78,ooo for the cytosol protein is identical with the molecular weight of polyribosomal P 78 as determined by SDS gel electrophoresis. (iii) The amino acid composition of cytosol P 78 closely resembles that of P 78 from mRNP. (iv) The reaction of cytosol P 78 with Sepharose-poly(A) was to some extent specific as has been shown in the case of cytosol P 78 from Ehrlich carcinoma ascites cells (7). A similar specificity of binding has been reported previously for endogeneous protein-polyadenylate structures (4, 13, 18).

It seems that the two proteins compared in this work are identical or at least highly related species. Thus, cytosol P 78 may be the precursor, or RNA-free form, of the mRNA-associated protein. Whether the free protein is involved, for example, in the process of nucleo-cytoplasmic transfer of mRNA, as has been suggested recently (2o) for mRNA-bound P 78, remains to be investigated. The fact that significant quantities of the free P 78 have been detected in cell nuclei (5) is in favour of this idea.

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