Glyceraldehyde-3-phosphate dehydrogenase is one of the three major RNA-binding proteins of rabbit reticulocytes

Alexei G. Ryazanov

Institute of Protein Research, Academy of Sciences of the USSR, 142292 Pushchino, Moscow Region, USSR

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One of the 3 major RNA-binding proteins of rabbit reticulocytes, a polypeptide of 36 kDa, is identified as glyceraldehyde-3-phosphate dehydrogenase (GAPD). This fact was deduced from the identity of molecular masses, one-dimensional peptide maps and isoelectric points of the 36 kDa protein and GAPD from rabbit muscle. It is concluded that GAPD can bind rather unspecifically different RNAs and polynucleotides. This means that GAPD, like other RNA-binding proteins, can form loose dynamic complexes with polyribosomes. Association of such a kind may be used for compartmentation of glycolysis near polyribosomes.

Glyceraldehyde-3-phosphate dehydrogenase *RNA-binding protein* *Polyribosome association* *Glycolysis compartmentation*

1. INTRODUCTION

In the cytoplasm of eucaryotic cells there is a fraction of proteins possessing nonspecific affinity for RNA (reviews [1,2]). It was shown for rabbit reticulocytes that this fraction contains both elongation factors, all aminoacyl-tRNA synthetases and most of the initiation factors of translation [3–6]. The set of polypeptides revealed after SDS electrophoresis of RNA-binding proteins of rabbit reticulocytes is relatively simple, consisting of 3 major polypeptides (96, 49 and 36 kDa) and several minor components [7]. The 96 and 49 kDa polypeptides were previously identified as elongation factors EF-2 and EF-1α, respectively [3], but the function of the 36 kDa protein remains unknown.

The key enzyme of the glycolytic pathway, glyceraldehyde-3-phosphate dehydrogenase (GAPD), is known to form complexes with nucleic acids [16–18]. Since the molecular mass of the GAPD subunits is nearly equal to that of the 36 kDa RNA-binding protein, it was tempting to determine whether these proteins are identical.

2. MATERIALS AND METHODS

GAPD (EC 1.2.1.12) from rabbit muscle was purchased from Reanal (Hungary), CNBr-activated Sepharose 4B from Pharmacia (Sweden), ampholines from LKB (Sweden), and V8 protease from *Staphylococcus aureus* from Miles Laboratories (England).

RNA-binding proteins from a ribosome-free extract of rabbit reticulocytes were obtained by affinity chromatography on Sepharose 4B with immobilized *E. coli* rRNA as in [7].

GAPD activity was followed spectrophotometrically by the appearance of NADH in the following buffer: 0.1 M glycine-NaOH, pH 8.9, 25°C, 1.0 mM NAD, 1.0 mM glyceraldehyde 3-phosphate, 5 mM sodium arsenate and 5 mM EDTA. One unit of activity corresponds to the amount of substrate in μmol transformed in 1 min at 25°C.

Protein was determined by the amido black staining technique on nitrocellulose filters [20].

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Table 1

Activity of GAPD in protein fractions adsorbed on RNA-Sepharose and unadsorbed

	Amount of protein (mg)	Total activity of GAPD (activity units)	Specific acativity of GAPD (activity units/mg)
Ribosome-free extract	18.5	1.75	0.1
Proteins adsorbed on RNA-Sepharose (RNA-binding proteins)	0.2	1.44	7.2
Proteins unadsorbed on rRNA-Sepharose	18.4	<0.1	<0.005

3. RESULTS

The reticulocyte ribosome-free extract was passed through the column with rRNA-Sepharose equilibrated with the following buffer: 10 mM Tris-HCl, pH 7.6 at 25°C, 10 mM KCl, 1 mM $MgCl_2$, 3.5 mM β-mercaptoethanol, 10% glycerol (rRNA-Sepharose was in excess for more complete adsorption of RNA-binding proteins). RNA-binding proteins were eluted with the same buffer containing 1 M KCl. GAPD activity was measured in the fractions of adsorbed and unadsorbed proteins. The results are listed in table 1. It can be seen from table 1 that GAPD was completely adsorbed on rRNA-Sepharose. The specific activity of GAPD in the fraction of RNA-binding proteins is about 7 units/mg. According to literature data, the specific activity of pure GAPD is about 50–150 units/mg. From these values one can calculate that in the fraction of RNA-binding proteins there is about 5–14% GAPD.

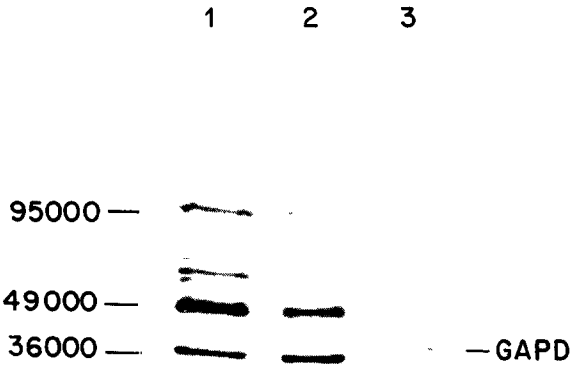

Fig.1. Electrophoresis of RNA-binding proteins and GAPD in 10% polyacrylamide gel with SDS. (1) RNA-binding proteins (40 μg), (2) RNA-binding proteins (35 μg) + GAPD (3 μg), (3) GAPD (3 μg).

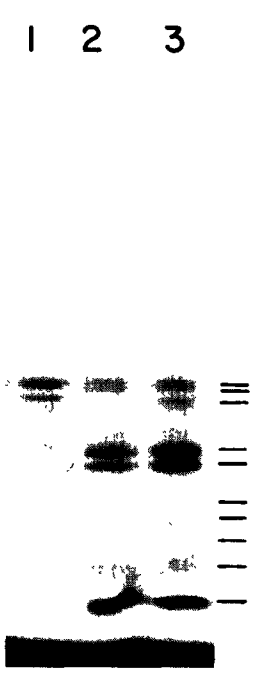

Fig.2. One-dimensional peptide maps of the 36 kDa protein and GAPD. The 36 kDa protein for peptide mapping and maps were prepared as in [11]. Electrophoresis was done as the second direction of 2-dimensional separation (see legend to fig.3). (1) GAPD (5 μg), 5 min incubation with V8 protease, (2) GAPD (5 μg), 15 min incubation with V8 protease, (3) the 36 kDa protein (10 μg), 15 min incubation with V8 protease. Peptides coinciding by mobility in both preparations are marked with dashes.

To establish which component of RNA-binding proteins corresponds to GAPD, preparations of GAPD from rabbit muscle and reticulocyte RNA-binding proteins were compared electrophoretically. As shown in fig.1, the mobility of the band corresponding to GAPD coincides with one of the 3 major RNA-binding proteins, the 36 kDa protein.

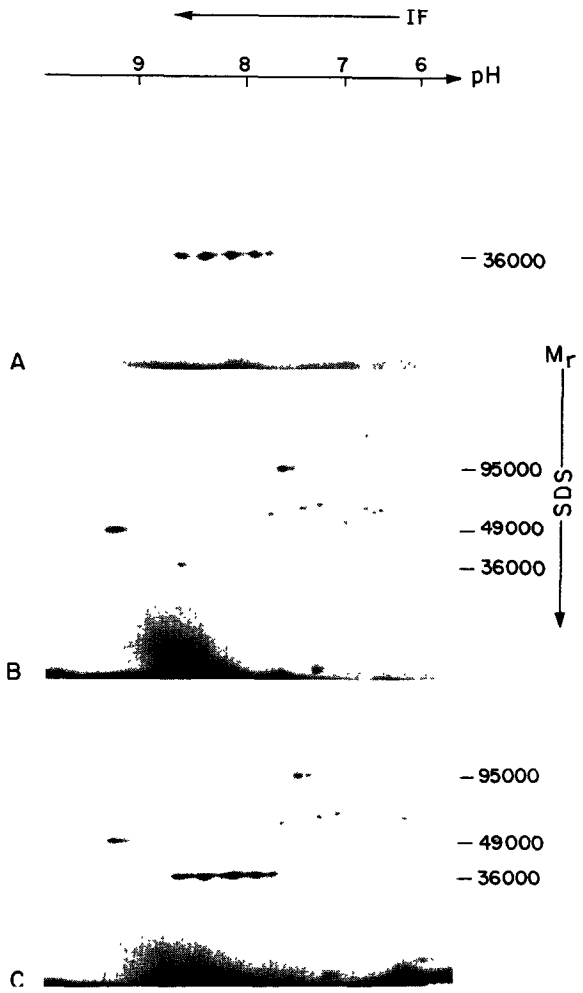

Fig.3. Two-dimensional separation of the preparation of RNA-binding proteins and GAPD. The procedure was carried out according to O'Farrell [12]. Ampholines 3–10 and 9–11 were used in a 3:1 ratio. The sample was applied from the anode. Electrophoresis in the second direction was carried out in a polyacrylamide gradient gel (10–22% acrylamide) in the presence of 0.1% SDS [19]. Proteins were stained with Coomassie G. (A) GAPD (18 μg), (B) RNA-binding proteins (30 μg), (C) RNA-binding proteins (30 μg) + GAPD (18 μg).

To prove finally that the 36 kDa protein is identical to GAPD, we made peptide maps of both proteins and compared their isoelectric points. One-dimensional peptide maps of GAPD and 36 kDa proteins are shown in fig.2. As can be seen, the maps of the 2 proteins are practically identical. During isoelectrofocusing GAPD separated into 5 spots (fig.3A). Fig.3 shows that one of 5 GAPD spots with the most basic isoelectric point coincides with the 36 kDa protein.

4. DISCUSSION

It was found previously that the fraction of RNA-binding proteins of rabbit reticulocytes contains nearly all proteins sufficient for the translational process [3–6], and that elongation factors EF-1 and EF-2 are 2 of the 3 major polypeptides of this fraction [3]. From the present work it follows that the third major polypeptide of 36 kDa is GAPD. This conclusion has been made on the basis of identity of molecular masses, one-dimensional peptide maps and isoelectric points of 36 kDa protein and GAPD from rabbit muscle.

According to [16–18], GAPD from mammalian tissues can bind single-stranded DNA, RNA and poly(A). In our laboratory it was shown that the 36 kDa protein can be adsorbed when a ribosome-free extract of rabbit reticulocytes is chromatographed on *E. coli* rRNA or poly(U) coupled to Sepharose [7]. These data mean that GAPD can bind rather unspecifically different RNAs and polynucleotides.

It has been shown for some RNA-binding proteins such as elongation factors and aminoacyl-tRNA synthetases that they can be loosely associated with polyribosomes due to their RNA-binding capacity [13–15]. It has been suggested that some RNA-binding proteins form dynamic constellations around polyribosome to provide for effective translation in the large and complicated eucaryotic cell [8–10]. I suggest that GAPD, like other RNA-binding proteins, can form loose dynamic complexes with polyribosomes.

It is well known that GAPD can form complexes with other glycolytic enzymes, including phosphoglycerate kinase [26,27], and different subcellular structures: membranes and cytoskeleton components (review [21]). Some data [22,23] indicate that association of GAPD with mem-

branes may promote, together with PGK, formation of a separate ATP pool which can be used by different ATP-dependent processes connected with the membrane. Analogously, association of GAPD with polyribosomes may promote formation of a pool of ATP and GTP for protein synthesis.

RNA-binding properties of GAPD can also be utilized for RNA unwinding during translation. An isoform of GAPD having the ability to lower the melting temperature of RNA and DNA was found in yeast [24]. Mammalian GAPD possesses the same ability [24]. On the other hand, it has been reported that in reticulocytes there is a special RNA-unwinding protein (or proteins) which is sufficient for normal translation [25]. This unwinding protein may be GAPD.

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