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Source: Zoological Science, 19(3) : 275-286

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.19.275
Biochemical and Immunohistochemical Studies on Tropomyosin and Glutamate Dehydrogenase in the Chicken Liver

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ABSTRACT—Recently, we have reported a novel tropomyosin (TM)-binding protein, glutamate dehydrogenase (GDH) and demonstrated by affinity column chromatography that chicken liver TM interacts with GDH in an ATP-dependent manner. To elucidate the physiological roles of the interaction between TM and GDH, we performed co-sedimentation assays of TM and GDH with F-actin, because it is known that TM exerts its physiological functions by associating with actin filaments. The results showed that TM and GDH co-pelleted with F-actin. GDH alone also co-precipitated with F-actin, but the amount of GDH sedimenting with F-actin was increased in the presence of chicken liver TM, suggesting that GDH is involved in the regulation of the actin cytoskeleton. We also prepared crude GDH from the nuclear and mitochondrial fractions obtained by subcellular fractionation of the chicken liver cells. Semi-nondenaturing 2D-PAGE revealed that partially purified GDH from the nuclear fraction was associated with TM, but not GDH from the mitochondrial fraction, suggesting preferential binding of TM to GDH. We determined the nucleotide sequence of chicken GDH cDNA and showed that the GDH transcript was widely expressed in the chicken organs. We examined the localization of TM and GDH by immunohistochemistry and revealed that they were distributed in the cytoplasm of the adult chicken liver. From these results, we propose two hypotheses on the physiological roles of the interaction between TM and GDH in nonmuscle cells.

Key Words: actin cytoskeleton, protein-protein interaction, intracellular localization, bifunctional protein, organelle movement

INTRODUCTION

It is well known that the actin-based cytoskeleton plays important roles in the intracellular physiological events such as muscle contraction, cell migration, cytokinesis, secretion, and so on. These multiple functions of the actin cytoskeleton are regulated by actin-binding proteins (Alberts et al., 1994). Tropomyosin (TM) is one of those proteins and binds to actin filaments along with their groove structures. Multiple TM isoforms, derived from four distinct genes by alternative splicing of their transcripts, are expressed especially in vertebrate nonmuscle cells and it is suggested that each TM has distinct function (Lees-Miller and Helfman, 1991; Pittenger et al., 1994; Lin et al., 1997).

It is revealed that TM plays important roles in the regulation of the actin cytoskeleton in nonmuscle cells. Disruption of the TM gene makes fission yeasts unable to divide into two daughter cells (Balasubramanian et al., 1992). Location of TM at the contractile ring, that mainly consists of actin and actin-binding proteins during cytokinesis, is shown by the study using cultured cells (Warren et al., 1995). Interestingly, overexpression of human TM3, a high-Mr TM isoform, in Ptk-2 cells induces perinuclear localization of organella, but not TM5a, a low-Mr TM isoform (Pelham et al., 1996). By injecting the TM-antibody and/or the caldesmon antibody into cultured cells, intracellular granule movement is greatly inhibited (Hegmann et al., 1989; 1991). Differential localization of TM isoforms is observed in cultured fibroblasts (Lin et al., 1988), LLC-PK1 cells (Temm-Grove et al., 1998), and synchronized NIH3T3 cells (Percival et al., 2000). Despite these evidences, the functional properties of TM in vertebrate nonmuscle cells are not elucidated well.

We have focused on TM-binding proteins, because the actin cytoskeleton is also modulated by the proteins such as caldesmon (Matsumura and Yamashiro, 1993), calponin (Winder and Walsh, 1997), and tropomodulin (Fowler, 1996). It is significant to reveal the precise functional properties of TM and its associated proteins in order to elucidate the regulation of the actin cytoskeleton. We searched for novel nonmuscle TM-binding proteins from the chicken liver by affinity column chromatography and demonstrated that glutamate dehydrogenase (GDH), that plays a central role in gluta-
mate metabolism, bound to the TM in an ATP-dependent manner (Akutsu et al., 2000), like caldesmon and calponin which associate with TM and F-actin in a Ca^{2+}-dependent fashion. GDH interacted with TM in the absence of ATP, but the association was inhibited by 0.1 mM ATP. ATP is one of the allosteric inhibitors for the enzymatic activity of GDH (Smith, 1975), and thus the GDH activity is also regulated in an ATP-dependent manner.

GDH is ubiquitously expressed in various organisms from bacteria to mammals (Smith et al., 1975). In vertebrates, it is widely accepted that GDH locates in the mitochondrial matrix. However, recent studies suggest that GDH also localizes at the nucleus (DiPrisco and Garofano, 1975; McDaniel, 1995), rough endoplasmic reticulum (Lee et al., 1999), and lysosome (Rajas and Rousset, 1993; Rajas et al., 1996). The GDH isoform at the lysosomal membrane possesses microtubule-binding ability in an ATP-dependent manner, suggesting the involvement of GDH in lysosomal movement and distribution in mammalian cells (Rajas et al., 1996).

In this study, we investigate the interaction of chicken liver TM and GDH to elucidate its physiological significance. Co-sedimentation assays of TM and GDH with F-actin suggest that GDH is involved in the regulation of the actin cytoskeleton. Semi-nondenaturing 2-D PAGE shows that GDH obtained from the chicken liver nuclear fraction interacts with TM. We determine the nucleotide sequence of chicken GDH cDNA and demonstrate that only a single transcript is expressed in the liver. We also show by immunohistochemistry that TM and GDH are distributed in the cytoplasm of hepatocytes. From these results, we propose two hypotheses on physiological roles of the interaction between TM and GDH in nonmuscle cells.

**MATERIALS AND METHODS**

**Preparation of chicken liver nuclear and mitochondrial fractions**

The liver was excised from the adult chicken, cut into small pieces with scissors, and then homogenized with Potter-Elvejem homogenizer at low speed (less than 1,000 rpm) in 4 volumes (V/W) of 0.25 M sucrose. The homogenate was centrifuged at 2,500×g, 2°C for 15 min using BECKMAN JA-20 rotor and the resultant supernatant and precipitate were pooled. For preparing the mitochondrial fraction, the supernatant was centrifuged at 5,000×g, 2°C for 10 min. The pellet was suspended in 0.25 M sucrose and centrifuged at 24,000×g, 2°C for 10 min. The resultant precipitate was dissolved in 0.25 M sucrose and centrifuged again. The precipitate was used as the chicken liver mitochondrial fraction. For preparing the nuclear fraction, the pellet obtained by centrifugation of the homogenate was suspended in 2.4 M sucrose and 2 mM MgCl_2 and subjected to ultracentrifugation at 100,000×g, 2°C for 1 hr using BECKMAN 50.2Ti rotor. The resultant precipitate was used as the chicken liver nuclear fraction. These nuclear and mitochondrial fractions were dissolved in 0.25 M sucrose and treated with cold acetone for several times. The resultant sediments were dried up thoroughly and stored at –20°C until use.

**Preparation of proteins**

All procedures were performed at 4°C unless otherwise mentioned. Chicken liver TM was prepared as described previously (Akutsu et al., 2000). Chicken GDH was partially purified from the nuclear and mitochondrial fractions obtained by subcellular fractionation of the chicken liver. Briefly, proteins were extracted from both the nuclear and the mitochondrial fractions with 1% Triton X-100, 0.05% cetyltrimethylammonium bromide, 10 mM K-PO_4, 1 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT, pH 7.5 and subsequently subjected to saturated ammonium sulfate fractionation. The resultant precipitates were dialyzed against Buffer I containing 10 mM K-PO_4, 1 mM EDTA, 0.1 mM PMSF, and 0.1 mM DTT, pH 7.5 for overnight. Dialyzed samples were loaded on HiTrap Blue affinity column (Amersham/Pharmacia Biotech) and bound proteins were eluted with graded KCl from 0 to 0.9 M and from 0.9 to 3 M and subsequently eluted with 3 M KCl. GDH-containing fractions were collected, concentrated, and then applied to HiLoad Superdex 200 prep grade gel filtration column (Amersham/Pharmacia Biotech). GDH-rich fractions were collected and used for the experiments described below. Chicken liver actin was prepared essentially according to Weir and Frederiksen (1980).

**Electrophoresis and Western blotting**

SDS-PAGE was performed according to Laemmli (Laemmli, 1970). Proteins on the gels were stained either with CBB-R250 or CBB-G250 (Neuhoff et al., 1988) or transferred to the nitrocellulose membrane according to Towbin et al. (1979). Western blotting was performed using the GDH-antibody (1:100, Rockland Inc.) as the primary antibody and goat TRITC-conjugated anti-rabbit IgG (H+L) (1:100, Jackson ImmunoResearch) as the second antibody. Two-dimensional gel electrophoresis (2-D PAGE) was performed by the Hitabayashi’s procedure (Hitabayashi, 1981). For semi-nondenaturing 2-D PAGE, 2-D PAGE was performed with the decreased urea concentration (2 M instead of 5 M) for preparing samples and agarose-IEF gels in the first dimension (Hitabayashi, 1981).

**Affinity column chromatography**

TM-affinity column chromatography was performed as reported previously (Akutsu et al., 2000). Purified chicken liver GDH was loaded on the TM-affinity column. Fractions eluted from the column were concentrated using Centricon-10 (Amicon) and subjected to 2-D PAGE. Proteins were stained with CBB-R or transferred to the nitrocellulose membrane (Towbin et al., 1979) for immunological detection with the GDH-antibody (1:100, Rockland, Inc.).

**Co-sedimentation assay**

Purified chicken liver TM, GDH, and F-actin were mixed well, incubated at room temperature for 2 hr in the buffer consisting of 0.1 M KCl, 6 mM MgCl_2, 20 mM Tris-HCl, pH7.5, 0.1 mM PMSF, and 0.1 mM DTT, and then ultracentrifuged at 270,000×g at 2°C for 20 min. Control experiments were also done in the same way using each or two of the three proteins. The supernatants and precipitates were loaded on the 12% SDS-polyacrylamide gel and proteins were stained with CBB-R.

**Nucleotide sequencing of chicken GDH cDNA**

Total RNA was extracted from the adult chicken liver using ISOGEN (NIPPON GENE) according to manufacturer’s protocol and synthesis of first strand cDNA was done using the oligo dt(12-18) (Amersham/Pharmacia Biotech) primer and SuperScript reverse transcriptase (GIBCO BRL). RT-PCR was performed using specific primers designed from nucleotide sequences of mammalian GDH, S1 (5'-CGACCCCAACTTCTTCAAGATGG-3'), S2 (5'-TRAYGTAGGCAGGCTGTCTCACAGG-3'), and A1 (5'-TRAYTG- TAGGCCAGGCTGTCTCACAGG-3'), and the primers designed from the partial nucleotide sequence of the GDH cDNA fragment amplified by RT-PCR with S1 and A1 primers, S2 (5'-CATTGGCTCCGTGAAGTTGATGTGC-3') and A2 (5'-GCTTGACCTGAGCATGGGC-3').
KOD Dash DNA polymerase (TOYOBO) was used for amplification of specific fragments. Step-down PCR was performed, by denaturing at 94°C for 1 min, annealing for 1 min, and extension at 74°C for 2 min. The annealing temperature was lowered in each 3 cycles by 4°C from 74°C to 58°C and at 54°C for last 20 cycles. RT-PCR products were subcloned into pBluescript II KS+ phagemid at Eco RV sites. Phagemids were isolated from transformed JM109 or DH5α competent cells by the alkaline-SDS method (Sambrook et al., 1989). GDH cDNA was subjected to cycle sequencing using ABI PRISM® Big-Dye Terminator (Perkin Elmer) according to the manufacturer’s protocol on ABI PRISM® 377 Sequencer (Perkin Elmer).

Preparation of chicken liver tissue sections

The adult chicken liver was cut into small pieces (5–10 mm) and fixed with 10% formalin in PBS for 3 hr. Subsequently, they were washed with water for overnight, dehydrated with graded ethanol followed by buthanol, and finally embedded in paraffin. Sections were prepared using a microtome in 5 µm-width and placed on poly-L-lysine-coated slide glasses. Sections were deparaffinized with xylene, rehydrated with graded ethanol, and washed with water. They were used for immunohistochemical staining.

Immunohistochemical staining of chicken liver tissue sections

Chicken liver tissue sections were blocked with 5% BSA in PBS at room temperature for 1 hr and incubated with the preimmune serum (1:100), the TM-antiserum (1:100), and the GDH antibody (1:100, Rockland Inc.) for 2 hr. Washing with PBS for 5 min 3 times, they were stained with goat TRITC-conjugated anti-rabbit IgG (H+L) (1:100, Jackson ImmunoResearch) for 2 hr. Subsequently, to eliminate background staining, sections were stained with 0.1% amidoblack and 0.7% acetic acid for 20 min. After washing with water, sections were enclosed with 90% glycerol in PBS. Fluorescence images were photographed with a combination of Nikon OPTIPHOT XF-Ph microscope and Nikon FX-35WA camera.

RESULTS

Interaction of chicken liver TM and GDH

As demonstrated in the previous study (Akutsu et al., 2000), chicken liver TM bound to GDH, an allosteric enzyme that catalizes transition of L-glutamate into 2-oxoglutarate (Smith et al., 1975). 2D-PAGE of eluates obtained by TM-affinity column chromatography showed that purified chicken liver GDH directly associated with TM (Fig. 1A). Western blotting using the GDH-antibody revealed that the protein eluted from the TM-affinity column cross-reacted with the antibody (Fig. 1B), confirming that the protein was GDH. GDH appeared as some spots on the 2D-PAGE gel.

Association of TM with GDH under the semi-nondenaturing condition

Semi-nondenaturing 2D-PAGE was performed in order to confirm the interaction of TM and GDH (Fig. 2). Since it is reported that GDH is located at the mitochondrial matrix (Smith et al., 1975) and also at non-mitochondrial organella such as the nucleus (DiPrisco and Garofano, 1975) and the rough endoplasmic reticulum (Lee et al., 1999), GDH was partially purified both from chicken liver nuclear and mitochondrial fractions and subjected to the semi-nondenaturing 2D-PAGE. When TM and GDH form the protein complex, they co-migrate on the semi-nondenaturing gel in the first dimension and align vertically on the SDS-polyacrylamide gel in the second dimension. When TM alone was subjected to semi-nondenaturing 2D-PAGE, faster-migrating minor components and slower-migrating major components were seen on the gel (Fig. 2A). The former was possibly composed of TM monomers and the latter of TM homodimers and/or heterodimers which were formed under the low concentration of urea in the first dimension. GDH was stuck on the top of the first dimension gels (Fig. 2B to E, arrowheads) and only a small portion of nuclear GDH entered the gel (Fig. 2B, double-arrowhead). However, almost all nuclear
GDH entered the gel in the presence of TM and co-migrated with TM (Fig. 2D, arrowhead accompanied with an asterisk). Mitochondrial GDH did not enter the gel even in the presence of TM (Fig. 2C and E). These results suggested that TM preferentially associated with the nuclear GDH.

**Co-sedimentation of chicken liver TM and GDH with F-actin**

Since it is widely accepted that TM plays its physiological roles by binding to actin filaments, we performed co-sedimentation assays of TM and GDH with F-actin. The resultant supernatants and precipitates were run on the 12% SDS-polyacrylamide gel (Fig. 3). While TM and GDH in the

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Fig. 2. Semi-nondenaturing 2D-PAGE with low concentration of urea in the first dimension. Semi-nondenaturing 2D-PAGE was performed using agarose gels containing 2 M urea instead of 5 M in the first dimension with chicken liver proteins of the following combinations. A, TM; B, nuclear GDH; C, mitochondrial GDH; D, TM+nuclear GDH; E, TM+mitochondrial GDH. Proteins were stained with CBB-G250. GDH hardly entered the first dimension gels (B to E, arrowheads), but a small amount of GDH prepared from the nuclear fraction did (B, double-arrowhead). In the presence of TM, nuclear GDH and TM co-migrated in the first dimension gel and aligned vertically on the second dimension gel (D, arrowhead accompanied with an asterisk), but mitochondrial GDH and TM did not (E).
absence of F-actin were in the supernatant after ultracentrifugation (Fig. 3D, F, and G), they co-pelleted with F-actin (Fig. 3A). Although TM or GDH alone co-sedimented with F-actin (Fig. 3B and C), the amount of GDH that co-precipitated with F-actin was slightly increased in the presence of chicken liver TM (Fig. 3A), suggesting that GDH is involved in the regulation of the actin cytoskeleton.

Expression of GDH mRNA and its nucleotide sequence

Recent studies have suggested the existence of two distinct GDH transcripts in human (Mavrothalassitis et al., 1988; Shashidharan et al., 1994). In order to see if several GDH transcripts are also expressed in the chicken, RT-PCR was performed using a template prepared from the adult chicken liver (Fig. 4A). A single fragment of approximately 1.4 kbp was specifically amplified with the S1 and A1 primer pair designed from the mammalian GDH cDNA sequences (Fig. 4A, SS, S1/A1) with no amplified product when either primer was used (Fig. 4A, S1, S2, A1, and A2) or when reverse transcriptase was not added (Fig. 4A, No). We newly designed S2 and A2 primers from the partial nucleotide sequence of the 1.4 kbp fragment and performed RT-PCR using S1, S2, A1, and A2 primers. There is no information of genomic and cDNA sequences of GDH in avians. The fragments of 1 kbp were specifically amplified with the S1/A2 and S2/A1 primer pairs and the fragment of 0.5 kbp with the S2/A2 primer pair (Fig. 4B). Note that no amplified product was obtained when one of the primers was used (Fig. 4B, S1, S2, A1, and A2) or when reverse transcriptase was not added (Fig. 4B, No). The 1.4 kbp fragment was also amplified by RT-PCR using templates prepared from the adult chicken spleen (Sp), brain (Br), skeletal muscle (Sk), and heart (He), thus suggesting that the GDH mRNA was widely expressed in the chicken organs (Fig. 4C).

Amplified products with S1/A2, S2/A2, and S2/A1 primer pairs were cloned and sequenced. The nucleotide sequence (1,436 bp) and its deduced amino acid sequence are shown in Fig. 5. It contained most of the open reading frame, but residual 5'- and 3'-sequences of the open reading frame and non-coding sequences were not determined in this study. Sequence identities of the chicken GDH to mammalian GDH including human GDH1 (Mavrothalassitis et al., 1988), GDH2 (Shashidharan et al., 1994), rat liver GDH (Amuro et al., 1989), and mouse brain GDH (Tzimagiorgis and Moschonas, 1991) were from 82 to 85% in nucle-
otides, and more than 90% in amino acids. Thus, GDH was highly conserved between chicken and mammals.

The α-helical (Fig. 5, underlined) domains were deduced from the secondary structure of bovine liver GDH (Peterson and Smith, 1999). It is likely that GDH can associate with TM via their α-helical domains, because it is suggested that troponin T, one of TM-binding proteins, interacts with TM through their α-helical regions (Pearlstone et al.,

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**Fig. 4.** RT-PCR for amplification of chicken GDH cDNA. A, RT-PCR was performed using the chicken liver total RNA as a template and S1 and A1 primers (S1/A1) which were designed from nucleotide sequences of mammalian GDH. RT-PCR was also carried out with either primer (S1 or A1) for negative control. Reverse transcriptase was added (SS) or not added (No) in the first strand synthesis. Amplified products were run on the 1% agarose gel. The fragment of approximately 1.4 kbp was specifically amplified. λ, λ DNA Hind III digest size marker. B, S2 and A2 primers were designed based on the partially determined nucleotide sequence of chicken GDH cDNA. Using S1, S2, A1, and A2 primers, RT-PCR was performed and the resultant products were subjected to agarose gel electrophoresis (S1/A2, S2/A2, S2/A1, S1, S2, A1, and A2, respectively). The fragments of 1 kbp (S1/A2 and S2/A1) and of 0.5 kbp (S2/A2) were specifically amplified. φ, φχ174 DNA Hae III digest size marker. C, RT-PCR was performed with S1 and A1 primers (S1/A1) and total RNA from the adult chicken organs as templates. Amplified products were subjected to agarose gel electrophoresis. Li, liver; Sp, spleen; Br, brain; Sk, skeletal muscle; He, heart. The fragment of approximately 1.4 kbp was amplified in all the organs.
Using the software of NetPhos 2.0, it was indicated that 16 serine, 6 threonine, and 5 tyrosine residues on the deduced amino acid sequence of chicken liver GDH (Fig. 5, boxed letters) were candidates for phosphorylation (Blom et al., 1999). Several spots of GDH seen on the 2D-PAGE gel (Fig. 1A) may be produced by phosphorylation, although the precise phosphorylation site(s) of chicken liver GDH has not been determined.

**Fig. 5.** Nucleotide sequencing of chicken GDH cDNA. Specifically amplified fragments with S1/A1, S1/A2, S2/A2, and S2/A1 primer pairs by RT-PCR were cloned and sequenced. The nucleotide sequence (1,436 bp) and the deduced amino acid sequence were aligned. Primers (S1, S2, A1, and A2) used in this experiment (bold letters) are denoted in the nucleotide sequence. The putative α-helical domains (underlined) and the predicted phosphorylation sites (boxed letters) are indicated in the amino acid sequence.
Immunohistochemical localization of TM and GDH in the chicken liver

To address localization of TM and GDH, paraffin-embedded adult chicken liver tissue sections were immunostained with the preimmune serum, the TM-antiserum, and the GDH-antibody, respectively (Fig. 6, ×100). Immunostaining results are shown with different regions of the tissue sections (Figs. 6 and 7). Although weak background staining

Fig. 6. Immunohistochemical observation of TM and GDH in the adult chicken liver. Formalin-fixed, paraffin-embedded adult chicken liver tissue sections were stained with the preimmune serum (1:100, A and B), the TM-antiserum (1:100, C and D), and the GDH-antibody (1:100, E and F), respectively. Goat TRITC-conjugated anti-rabbit IgG (H+L) antibody (1:100, Jackson ImmunoResearch) was used as the secondary antibody. BV and BD indicate the blood vessel and the bile duct, respectively. Bar, 50 µm; magnification, ×100.
TM and GDH in the Chicken Liver

was seen in the sections that were stained with the preimmune serum (Fig. 6A and B), immunostaining with the TM-antiserum and the GDH-antibody was distinctive from that with the preimmune serum. TM was localized in the peripheral regions of the sinusoids and bile ducts (BD) (Fig. 6C and D). Note that TM was also located at blood vessels (data not shown). GDH was distributed in the cytoplasm of hepatocytes, but not at the peripheries of sinusoids, bile ducts, and blood vessels (BV) (Fig. 6E and F). In order to demonstrate precise location of TM and GDH in the chicken liver, immunostained sections were observed at the higher (×250) magnification (Fig. 7). TM was localized weakly in the cytoplasm of hepatocytes and the peripheral regions of the sinusoids (SS) (Fig. 7A and B). GDH was distributed in the cytoplasm of hepatocytes with dotted structures (Fig. 7C and D).

DISCUSSION

In this study, we showed by co-sedimentation assays that GDH co-precipitated with F-actin and the amount of GDH sedimenting with F-actin was slightly increased by TM. It suggests that GDH is involved in the regulation of the actin cytoskeleton by binding to TM. It is not surprising that GDH co-sedimented with F-actin in the absence of TM, because several known TM-binding proteins, such as caldesmon (Matsumura and Yamashiro-Matsumura, 1993), calponin (Winder and Walsh, 1997), and tropomodulin (Fowler, 1996), bind both to TM and F-actin. It is known that caldesmon increases the association of TM with actin filaments, stabilizing the actin cytoskeleton (Yamashiro-Matsumura and Matsumura, 1988; Pittenger et al., 1995) and that the F-actin pointed end-capping activity of tropomodulin is enhanced in the presence of TM (Fowler, 1996). Thus, it is possible that the association of GDH with TM stabilizes the actin cytoskeleton. Alternatively, it is also possible that GDH utilizes the actin cytoskeleton via TM as a scaffold by exerting its enzymatic activity at particular regions of the cytoplasm in nonmuscle cells.

Semi-nondenaturing 2D-PAGE showed that TM associ-
ated with nuclear GDH, but not with the mitochondrial GDH, suggesting preferential binding of TM to GDH. It has been widely accepted that GDH is localized in the mitochondrial inner space and also shown by recent biochemical and immunocytochemical studies that non-mitochondrial GDH isoforms are expressed in mammalian tissues. However, cloning and sequencing of chicken liver GDH cDNA in this study revealed that a single transcript was specifically amplified by RT-PCR. It is possible that other GDH transcripts could not be detected due to their small quantities in the adult chicken liver or that they have substantially different nucleotide sequences and thus could not be amplified by RT-PCR with primers used in this study. Alternatively, differences among transcripts may be restricted to the their 5'- and/or 3'-coding regions, sequences of which were not determined in this study, as rat liver microsomal aldehyde dehydrogenase cDNA has no mitochondrial signal sequence at the 5'-terminal region compared with the mitochondrial aldehyde dehydrogenase cDNA, but has an additional sequence at the 3'-terminal region encoding a hydrophobic segment, that is required for the precise location of the protein at the endoplasmic reticulum (Miyauchi et al., 1991; Masaki et al., 1994). Structural analyses of the chicken GDH gene(s) are useful to examine whether multiple transcripts can be produced or not. Human GDH isoforms, designated as GDH1 (Mavrothalassitis et al., 1988) and GDH2 (Shashidharan et al., 1994), are encoded by distinct genes. Our studies showed that several TM isoforms were present in the chicken liver. Therefore, it is interesting to examine specific interactions between TM isoforms and possible GDH isoforms.

Immunohistochemical staining of the chicken liver suggests that TM and GDH were localized in the cytoplasm of hepatocytes, but their precise localization could not be revealed in this study. It seems that the dotted structures detected with the GDH-antibody may be mitochondria. It has not been reported that TM is distributed in the mitochondrial inner space, and such dotted structures were not detected with the TM-antiserum. Several biochemical and immunocytochemical studies have indicated that GDH is localized at the lysosomal membrane (Rajas and Rousset, 1993; Rajas et al., 1996), nucleus (DiPrisco and Garofano, 1975; McDaniel, 1995), and rough endoplasmic reticulum (Lee et al., 1999). It is conceivable that a membrane-bound form of GDH can bind to the actin cytoskeleton via TM. It has been revealed that TM is involved in intracellular granule movement in cultured cells (Hegmann et al., 1989) and budding yeasts (Liu and Brescher, 1992), and that human TM3 isoform is related to organelle movement and distribution (Pelham et al., 1996). Thus, it is possible that an organelle movement is regulated by the interaction between TM and GDH. It is intriguing to examine by double staining of chicken liver tissue sections and cultured cells using the GDH-antibody and a specific antibody against an organelle membrane-bound protein in order to reveal GDH indeed locates at the organelle membrane.

We showed in the previous study (Akutsu et al., 2000) that GDH interacted with TM in the absence of ATP, an allosteric inhibitor of GDH enzymatic activity, but the interaction was inhibited by 0.1 mM ATP. From our previous and present results, we propose two hypotheses on physiological roles of the interaction between TM and GDH in nonmuscle cells (Fig. 8). First, GDH associates with TM on actin filaments in the absence of ATP. In the absence of the allosteric inhibitor, GDH stabilizes its filamentous structure together with TM, or exerts its enzyme activity on the actin cytoskeleton at particular regions of the cytoplasm because of the absence of the allosteric inhibitor. On the contrary, GDH dissociates from the actin filaments and its enzymatic activity is prevented in the presence of ATP (Fig. 8A). Sec-
ond, the organelle membrane-bound GDH interacts with actin filaments via TM in the absence of ATP, and thus the organelle is anchored at limited regions of the cytoplasm in nonmuscle cells. In the presence of ATP, the association of GDH with the actin filaments is prevented. Instead, an actin-based motor protein such as myosin I, which is known to locate at the organelle membrane (Mermall et al., 1998), can interact with actin filaments utilizing ATP as an energy source, resulting in movement of the organelle along the actin cytoskeleton to particular portions of the cytoplasm in nonmuscle cells (Fig. 8B).

The present results invoke bifunctional proteins. Translation elongation factor-1α catalyzes the GTP-dependent binding of aminoacyl tRNA to the ribosome acceptor site in the peptide elongation phase of protein synthesis and it also participates in the regulation of microtubules and actin filaments (Condeelis, 1995). Aldolase is a glycolytic enzyme and also recognized as an actin-binding protein (Arnold et al., 1968). Its enzymatic activity is modulated by interacting with actin filaments, and the interaction is prevented in the presence of its substrate, fructose 1, 6-bisphosphate (Walsh et al., 1977). Crystallins are known as major components of the lens. αB-crystallins are ubiquitously expressed in vertebrate tissues and exert chaperone activities (Graw, 1997). GDH catalyzes transition of L-glutamate into 2-oxoglutarate and participates in the regulation of actin filaments and microtubules (Rajas and Rousset, 1993; Rajas et al., 1996). Therefore, it is suggested that GDH is adjoined to members of bifunctional proteins.

REFERENCES


Pittenger MF, Kazzaz JA, Helfman DM (1994) Functional properties of...


(Received December 3, 2001 / Accepted December 17, 2001)