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DIFFERENT EXPRESSION OF RAT ALDOLASE A mRNA IN THE SKELETAL MUSCLE AND ASCITES HEPATOMA CELLS

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Rat aldolase A mRNAs from the skeletal muscle and ascites hepatoma cells are compared by RNA blot hybridization. The hepatoma AH60C and AH130 mRNA are about 1600 bases long, some 50 bases longer than the skeletal muscle transcript. Aldolase A enzymes encoded by these mRNAs, however, are indistinguishable in electrophoresis on cellulose polyacetate strips and two-dimensional polyacrylamide gel. In addition, comparison of the thermal stability of hybrids between the mRNAs from the skeletal muscle and hepatoma cells and a cDNA probe (pRAAM83) made from the aldolase A mRNA of the skeletal muscle indicate that the mRNAs have extremely high sequence homology, at least, within the most part of coding region in addition to the 3' noncoding region. These findings indicate that these mRNA may be transcribed by the use of different promoter sites and the common structural gene.

The resurgence of fetal isozyme in cancer, such as aldolase, pyruvate kinase, and lactate dehydrogenase, is now generally accepted (1,2). In the case of fructose-1,6-diphosphate aldolase, the muscle type enzyme (aldolase A), which is nearly absent in adult liver but is present in fetal liver, reappears in a fast-growing hepatoma instead of the liver type enzyme (aldolase B) (1,2). It is also confirmed that aldolase A originated from a hepatoma such as Novikoff hepatoma or primary hepatoma induced by 3'-methyl-1,4-dimethylamino azobenzene is indistinguishable from the enzyme isolated from the normal skeletal muscle on their catalytic activities, physicochemical properties and amino acid compositions (3,4).

Very little is known, however, about the mechanism which might operate in switching the isozyme patterns from the adult to the fetal type in fast-growing hepatoma cells. Studying the gene expression of rat aldolase A at the transcriptional level, we have analyzed the molecular size and sequence homology between aldolase A mRNAs isolated from the skeletal muscle and hepatoma, and compared the aldolase A enzymes as well.

Abbreviations used: cDNA; complementary DNA, DBM paper; diazobenzyloxymethyl paper, 1x Denhardt; 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, kb; kilobase, NADH; nicotinamide adenine dinucleotide (reduced), NEPHGE; non-equilibrated pH gradient gel electrophoresis, oligo(dT) cellulose; oligodeoxythymidylate cellulose, poly(A) RNA; polyadenylated RNA, SDS; sodium dodecyl sulfate, SSC; standard saline citrate.

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Here we report that these mRNAs are different in size in spite of having extremely high sequence homology and encoding the same enzyme in different tissues.

MATERIALS AND METHODS

Chemicals: A cellulose polyacetate membrane, nitrocellulose filter and DBM paper were obtained from Schleicher and Schuell. Fructose 1,6-diphosphate and NADH were purchased from Boehringer and Mannheim. Guanidinium isothiocyanate was obtained from Fluka. Ampholines were purchased from LKB. Oligo-(dT) cellulose was obtained from Collaborative Research.

Enzymes and cells: α-glycerophosphate dehydrogenase and triose phosphate isomerase were obtained from Boehringer and Mannheim. Rat ascites hepatoma AH60C and AH130 cells, originally induced by 3'-methyl-1,4-dimethylamino azobenzene (5) were provided by Dr.Hideya Endo of Kyushu University and maintained by serial transplantation.

Plasmid DNAs: pRAAM83, a cDNA clone of rat aldolase A mRNA and pRAB3031, a cDNA clone of rat aldolase B mRNA were prepared in our laboratory (6,7).

Electrophoresis: Electrophoresis on cellulose polyacetate strips and staining for aldolase activity were carried out as described by Susor et al. (8). Two-dimensional gel electrophoresis (NEPHGE-SDS) was performed by the method of O'Farrell et al.(9).

Purifications of rat aldolases and the mRNAs: Purifications of aldolase A from rat skeletal muscle and hepatoma and of aldolase B from rat liver were performed according to Gracy et al.(3). For whole cell RNA isolation, tissues were ground for several minutes by a Waring Blender in liquid nitrogen, homogenized in 5 volumes of 50mM Tris-HCl buffer, pH7.6 containing 4M guanidinium isothiocyanate, 140mM β-mercaptoethanol, 2% sarkosyl and 10mM EDTA in a glass/Teflon homogenizer, and extracted with phenol-chloroform at 60°C (10). Extracted total RNAs were applied on oligo-(dT) cellulose column and polyadenylated RNAs were enriched from the total RNAs.

RNA blotting and hybridization: Poly(A) RNAs were electrophoretically separated on a vertical formamide/1% agarose slab gel, and transferred to a nitrocellulose filter according to the method in Thomas (11). RNA immobilized on a filter was hybridized to nick-translated probe [^{32}P]-cDNA as indicated in Fig.1.

Melting experiment of the DNA-RNA hybrids: Poly(A) RNAs prepared from skeletal muscle and ascites hepatoma AH60C and AH130 cells were covalently attached to DBM-paper and hybridized with a nick-translated probe [^{32}P]-cDNA. Filters were heated at the indicated temperature for 5 min in 400μl of 6.67xSSC. The radioactivities released at each temperature were determined and plotted cumulatively.

RESULTS

Size of aldolase A mRNAs: In order to compare the size of aldolase A mRNA from a rat skeletal muscle and hepatomas AH60C and AH130, RNAs were blotted on a nitrocellulose filter from the agarose gel after electrophoresis, hybridized to a nick-translated cDNA probe and then autoradiographs were obtained(Fig.1A). Unexpectedly, the size of aldolase A mRNA from the skeletal muscle was smaller than that from the hepatoma and was estimated to be 1.55kb for skeletal muscle mRNA and 1.60kb for hepatoma mRNAs, respectively. There was no difference in size between AH60C and AH130 mRNAs. Furthermore, the size of aldolase A mRNA being expressed in a normal adult liver was the same as to the hepatoma mRNAs (Fig.1A, lane 4). Although liver RNA hybridized to both cDNA probes for

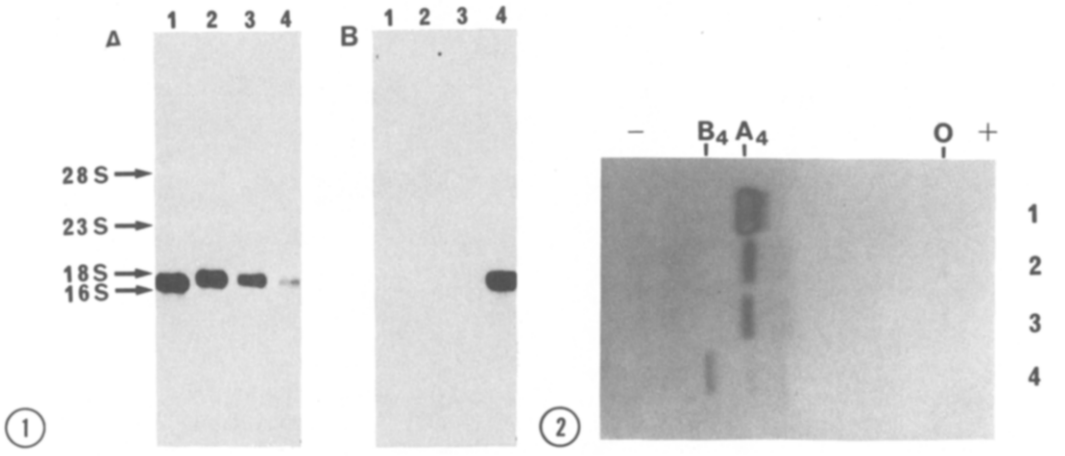

Fig.1 The detection of aldolase A mRNAs in different tissues. Poly(A) RNA from skeletal muscle, ascites hepatoma and adult liver tissues was fractionated on a formamide/1% agarose gel. The RNAs were transferred to nitrocellulose filter and hybridized to nick-translated [^{32}P]-pRAAM83 cDNA or pRAB3031 cDNA probe in a solution containing 50% formamide, 5xSSC, 50mM sodium phosphate pH6.5, 250µg/ml denatured salmon sperm DNA, 50µg/ml yeast RNA, 1x Denhardt and 0.1% SDS at 42°C. After hybridization, filters were washed in 0.1xSSC, 0.1%SDS, at 62°C. (A) Lane 1, 0.5µg of skeletal muscle poly(A) RNA; Lanes 2 and 3, 1µg of AH130 and AH60C poly(A) RNA, respectively; Lane 4, 5µg of adult liver poly(A) RNA. [^{32}P]-pRAAM83 was used as a cDNA probe. (B) Lanes 1,2 and 3, 5µg of skeletal muscle, AH130 and AH60C poly(A) RNA, respectively; Lane 4, 1µg of adult liver poly(A) RNA. [^{32}P]-pRAB3031 was used as a cDNA probe. Rat rRNA and Escherichia coli rRNA were used as size markers.

Fig.2 Patterns of aldolase activity of adult rat tissues after cellulose polyacetate electrophoresis. Electrophoresis and activity staining for aldolase were performed as indicated (8). The crude extracts were prepared from the following tissues of Donryu rat. 1.skeletal muscle, 2.AH130 cells, 3.AH60C cells and 4.adult liver.

aldolase A and B, the RNA hybridized to the former cDNA probe appears to represent the aldolase A mRNA since mRNAs from skeletal muscle and hepatoma cells hybridized only to the aldolase A cDNA probe, but did not to the aldolase B cDNA probe under the same stringent hybridization conditions.

Aldolase isozymes in skeletal muscle and hepatomas: We confirmed that aldolase A almost completely substituted for aldolase B enzyme in the poorly differentiated hepatoma as reported earlier (3,4). Electrophoresis of extracts from skeletal muscle, adult liver and ascites hepatomas AH60C and AH130 was carried out on cellulose polyacetate strips, followed by staining for aldolase activity. As shown in Fig.2, the aldolase B migrated rapidly toward the cathode while the enzyme from ascites hepatomas showed a much lower mobility corresponding to that of the muscle type aldolase.

Two-dimensional gel electrophoresis of these enzymes indicated that aldolase A from the skeletal muscle gave a spot at a position different from that of aldolase B (Fig.3A), whereas under the same conditions muscle and hepatoma aldolases completely comigrated through the first and second dimension gels and

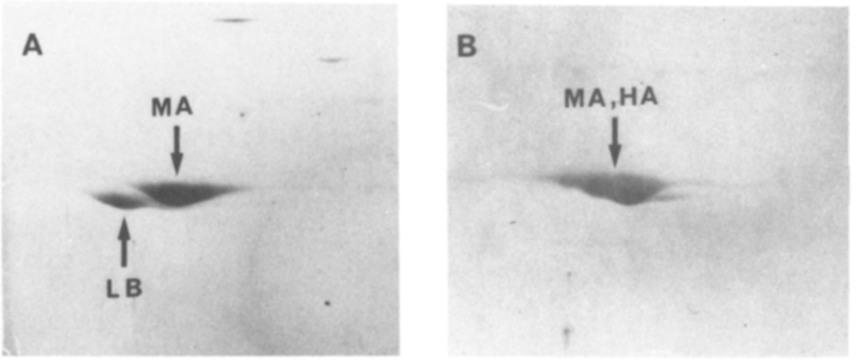

Fig.3 Two-dimensional gel electrophoresis of purified aldolases from rat liver, skeletal muscle and ascites hepatoma AH60C. The NEPHGE-SDS system (9) was used in this experiment. The first dimension of electrophoresis was performed for 5 hr at 400V at room temperature by using Amplholines of PH ranges 3.5-10 (0.2%) and 7-9 (0.8%). In the second dimension, a 10-16% exponential gels were used. A. Rat liver aldoase B (LB) and skeletal muscle aldolase A (MA). B. Rat aldolase A of skeletal muscle (MA) and ascites hepatoma AH60C (HA). Gels were shown with acid end to the right and SDS electrophresis proceeding downward.

did not give discrete spots which are expected if their molecular size and/or isoelectric points are significantly different each other (Fig.3B). These results, together with the data shown in Fig.2, indicate that the skeletal muscle and hepatoma enzymes are identical and are in agreement with the previous conclusions described by others (3,4).

Sequence homology between skeletal muscle and hepatoma mRNAs: In order to clarify the structure relationship between the skeletal muscle and hepatoma aldolase A at the mRNA level, the degree of mRNA sequence homology was compared. For the purpose the thermal stabilities of the hybrids between the nick-translated [^{32}P]-cDNA probe (pRAAM83) and poly(A) RNAs from the muscle and hepatoma cells were determined and the melting curves were plotted (Fig.4). The recombinant cDNA probe, pRAAM83 plasmid, was not a full length cDNA which spans about 80% of the expected total length from the 3' end and deletes the 5' noncoding region completely and part of the coding region near the N-terminus of the enzyme. The melting curves of the hybrids between cDNA probe and poly(A) RNAs from these different sources completely coincided and the melting temperature was 85^{o}C for these all hybrids. The results of the thermal stability indicate that aldolase A mRNAs expressed in these different tissues have the same primary structure (nucleotide sequence), at least, within the region which hybridizes to the cDNA probe and indicate further that each mRNA might be transcribed from the common structural gene. The results of both partial nucleotide sequencing and cleavage mapping which were performed with the cDNA clones for the skeletal muscle and ascites hepatoma aldolase A mRNAs strongly support these results (data not shown).

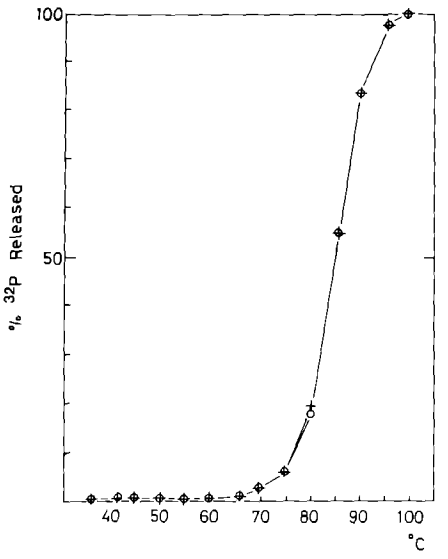

Fig.4 Melting curves of aldolase A cDNA-RNA hybrids. Poly(A) RNAs prepared from different tissues were bound to DBM paper and hybridized with nick-translated [^{32}P]-pRAAM83 cDNA at 42°C in the same solution used in the hybridization experiments described in Fig.1 except for 50μg/ml yeast RNA. Filters were heated up at various temperatures and the radioactivities released from the filters were counted in a scintillation counter. It is plotted in the percentage of the sum of the [^{32}P] -radioactivities released at each templeratrue. +—+: melting curves of hybrids between skeletal muscle poly(A) RNA and [^{32}P]-pRAAM83 cDNA. o—o: melting curves of hybrids between AH60C poly(A) RNA and [^{32}P]-pRAAM83 cDNA. The melting temerature (Tm) determined from the curves was 85°C for both DNA-RNA hybrids.

DISCUSSION

In this work we could clarify that aldolase A mRNA from the fast-growing hepatoma is unexpectedly different in size from that of aldolase A in the skeletal muscle. From the difference in mobility in the RNA blot hybridization, it was estimated that hepatoma mRNAs are about 50 nucleotides longer than the skeletal muscle mRNA (Fig.1A).

Several laboratories have reported that the aldolase B, which is predominant component as aldolase isozymes in normal adult liver, is replaced by aldolase A in a fast-growing hepatoma, concluding that the aldolase A expressed in the tumor cells is the same protein as the muscle type enzyme from skeletal muscle (3,4). As described in this paper, the results obtained with the electrophoresis on cellulose polyacetate strips (Fig.2) and the two-dimensional polyacrylamide gel electrophoresis (Fig.3) are compatible with the previous observations (3,4). This conclusion was also indirectly confirmed by the experiments on the mRNA level which compared the thermal stability of hybrids between a cDNA probe and mRNAs (Fig.4) and by other unpublished observations as descrebed above.

Despite aldolase A of a hepatoma and skeletal muscle being identical, their mRNAs appear to differ in size. Therefore, these two mRNAs might be produced

by the use of different promoters and the differential processing at the 5' and/or 3' noncoding regions of a single transcript. There is also another possibility that these mRNAs may have the different length of poly(A) tail. Our results is in favor of the former because aldolase cDNA used as a probe in the thermal stability experiments spans the entire 3' noncoding sequence and nearly full coding sequence except for the 5' noncoding sequence and short strech of N-terminal region of the aldolase enzyme, and because the size difference of these mRNAs persists after removal of poly(A) tail (unpublished observation). The synthesis of different mRNAs from a single gene by the use of alternative promoter was described in mouse α-amylase-1 gene (12), the invertase gene of yeast (13) and Drosophila melanogaster alcohol dehydrogenase gene (14).

It is unclear whether the mRNA synthesized in hepatoma is specific for tumor cells. Since the aldolase A mRNAs expressed in adult liver and hepatoma cells did not differ in size (Fig.1A, lane 4), these transcripts might be identical. Furthermore, these results appear to reflect that the transcriptional organization of aldolase A gene in skeletal muscle might differ from these in the normal adult liver and hepatoma.

We have found a good correlation between the appearance and the disappearance of aldolase mRNA and those of the corresponding types of enzymes in normal and hepatoma cells (see Fig.1 and 2). In ascites hepatoma cells we could neither find any amount of aldolase B enzyme activity which is normally found in adult liver nor aldolase B mRNA (compare Fig.1A and B, lanes 2 and 3, and Fig.2, lanes 2 and 3). In the cell extracts, we could only find aldolase A mRNA as was observed with aldolase A activity. Likewise, the similar relationship has been obtained with the mRNA and enzyme levels in normal adult liver or skeletal muscle. It is evident from these observations that the expression of an aldolase gene would be, at least, under the transcriptional control.

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