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NUCLEOTIDE SEQUENCE OF A cDNA CLONE FOR HUMAN ALDOLASE: A MESSENGER RNA IN THE LIVER

Minoru Sakakibara*, Tsunehiro Mukai, and Katsuji Hori**

Department of Biochemistry, Saga Medical School,
Saga 840-01, Japan

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Nearly complete cDNA clones for human aldolase A mRNA were isolated from human liver cDNA library and the nucleotide sequence determined. Using the cDNA clone as a probe the length of human aldolase A mRNAs, isolated from the skeletal muscle, liver and placenta tissues, was measured by RNA blotting and estimated to be 1,600 nucleotides for skeletal muscle mRNA and 1,700 nuceotides for both the liver and placenta mRNAs, indicating that different species of mRNA coding for human aldolase A were expressed in the different tissues.

The glycolytic enzyme fructose-1,6-diphosphate aldolase (EC 4.1.3.13) consists of three genetically distinct isozymes; aldolase A (muscle type), aldolase B (liver type) and aldolase C (brain type). Their expression is tissue-specific and regulated during the course of development (1). Therefore, aldolase isozyme seems to be an useful material to elucidate a regulatory mechanism of tissue-specific or developmental gene expression. For this purpose we have first carried out the structural analysis of aldolase isozyme genes and their transcripts. Together with the study on rat aldolase genes(2-5), we have also begun to analyze the structure of human aldolase A and B genes in order to elucidate the molecular mechanism of inborn errors of metabolism by aldolase A and B deficiency, such as hereditary nonspherocytic anemia (6) and hereditary fructose intolerance (7, 8), and also to compare the genes, especially the regulatory region of the genes, in different animals for understanding the molecular mechanism of gene expression. Recently, several groups have reported the partial or nearly complete nucleotide sequence of human aldolase B mRNA (9-11).

We present here the characterization of cDNA clones for human aldolase A mRNA which were screened from the liver cDNA library. We describe the structural relatedness among aldolase A mRNAs of human and other species (12) since this is the first complete analysis on the structure of human aldolase

* On leave of absence from the Laboratory of Biochemical Research, Toyo Jozo Co.Ltd., Oohito, Shizuoka 410-23, Japan.

** To whom correspondence and reprint requests should be addressed.

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A mRNA and allows us to compare them. We also present evidence supporting the presence of different species of aldolase A mRNAs expressed in various human tissues in a tissue specific manner.

MATERIALS AND METHODS

Materials: Avian myeloblastosis virus (AMV) reverse transcriptase was purchased from Life Science Inc. Restriction enzymes and other enzymes were from Takara Shuzo Co., Ltd., Toyobo Co.Ltd. and PL Biochemicals Inc. Radioactive nucleotides were obtained from Amersham.

Isolation of aldolase A cDNA clones from human liver cDNA library: The human liver cDNA library was kindly supplied by Dr.Sigetada Nakanishi of Kyoto University School of Medicine. Double stranded cDNA was prepared from human adult liver poly(A)$^+$ RNA according to the method of Okayama-Berg (13) and transformed to Escherichia coli K12 strain DH1. Colonies were grown in an array on nitrocellulose filters (2,000 colonies per filter) and screened by colony hybridization (14) using a rat aldolase A cDNA, pRAAM83 (4), which was radiolabeled by nick-translation (15). Hybridization was carried out at 60°C as described (16).

RNA blottings: Poly(A)$^+$ RNAs were extracted from human tissues, transferred to a nitrocellulose filter (17) after electrophoresed on 1% agarose gel containing formaldehyde (18) and hybridized to [^{32}P]-labeled HinfI-AluI fragment, 220 base pairs (bp) of 3' portion of pHAAL116-3 as described(16).

Sequence analysis: Nucleotide sequence analysis was performed by the dideoxy technique of Sanger et al.(19). DNA fragments were cloned into M13mp8 or M13mp11 as described by Messing (20) with some modifications.

RESULTS

Isolation and identification of human aldolase A cDNA clones: Aldolase A cDNA clones were isolated from a human liver cDNA library using a rat aldolase A cDNA, pRAAM83 (4) as a hybridization probe during an attempt to isolate human aldolase B cDNA clones from the same cDNA library since aldolase A was known to be more or less expressed in adult liver tissues (21). Twelve positive clones, 0.01% of total transformants, were obtained from 1.4×10^5 colonies. The typical cDNA clones, pHAAL116-3 and pHAAL207-4, and their relations to the mRNA along with the restriction map and the sequencing strategy are shown in Figure 1. The cleavage maps of these independent clones completely coincide and were confirmed to be similar to those of rat aldolase A cDNAs (4). One of these clones, pHAAL116-3 was selected for detailed characterization. From the amino acid sequence deduced from the nucleotide sequence we concluded that this clone is a human aldolase A cDNA as described in the following section.

Nucleotide sequence: The pHAAL116-3 was sequenced according to the strategy shown in Figure 1 using the dideoxy technique (19). The sequence of the cDNA insert which included 167 nucleotides for the 5'-noncoding, 1,092 and 205 nucleotides for the coding and 3'-noncoding regions, respectively, was determined. The nucleotide sequence of pHAAL116-3 is presented in Figure 2 along with the amino acid sequence deduced from a possible open reading frame. The deduced aldolase A protein sequence included 363 amino acids,

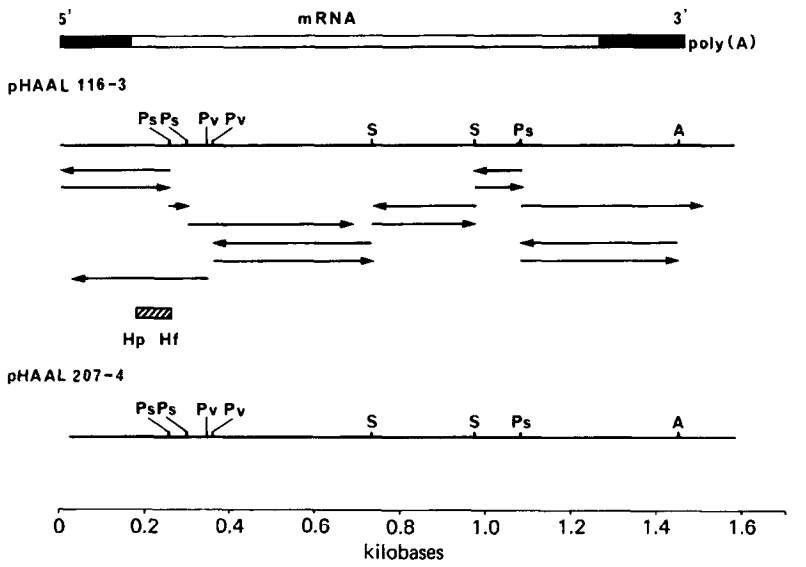

Figure 1. Restriction endonuclease cleavage maps of cloned aldolase A cDNA and sequencing strategy. The presumed structure of aldolase A mRNA from human liver is shown as an open box (coding region) and solid boxes (noncoding region) at the top of the figure. The restriction sites indicated are: AluI (A), HinfI (Hf), HpaII (Hp), PstI (Ps), PvuII (Pv) and Sau3AI (S). AluI site in the figure shows only site used for sequencing. The sequencing strategy is indicated by horizontal arrows. The amount of the cDNA sequence determined in both directions was 98%. The DNA fragment (HpaII- HinfI) used for the primer extension is presented by ▨.

excluding the initiating methionine and was found to begin with a proline as the N-terminal amino acid residue since the mature enzyme was known to lack the methionine (22). The amino acid sequence deduced from the nucleotide sequence was completely consistent with the partial amino acid sequence of human skeletal muscle aldolase A reported by Freemont et al.(22). Thus we concluded the pHAAL116-3 clone was a human aldolase A cDNA clone.

We compared the deduced amino acid sequence of human liver aldolase A with those of the rabbit (12) and rat (4) skeletal muscle aldolases A. The amino acid sequence homology between the human and rabbit aldolases A and between the human and rat ones was 98.3% (357/363) and 96.4% (350/363), respectively. On the other hand, the sequence homology between human aldolases A and B (23) and between human aldolase A and rat aldolase B was 69.4% (252/363) and 69.1% (251/363), respectively (3). These results indicate that the sequence homology in the same type aldolase isozymes among the different species is higher than that in the different type aldolase within a species.

There is also a high sequence homology at the 3'-noncoding region among these three mRNA species, although they are significantly different in length. In contrast, human liver aldolase A mRNA is considerably different from the rabbit and rat skeletal muscle aldolase A mRNAs at the 5'-noncoding

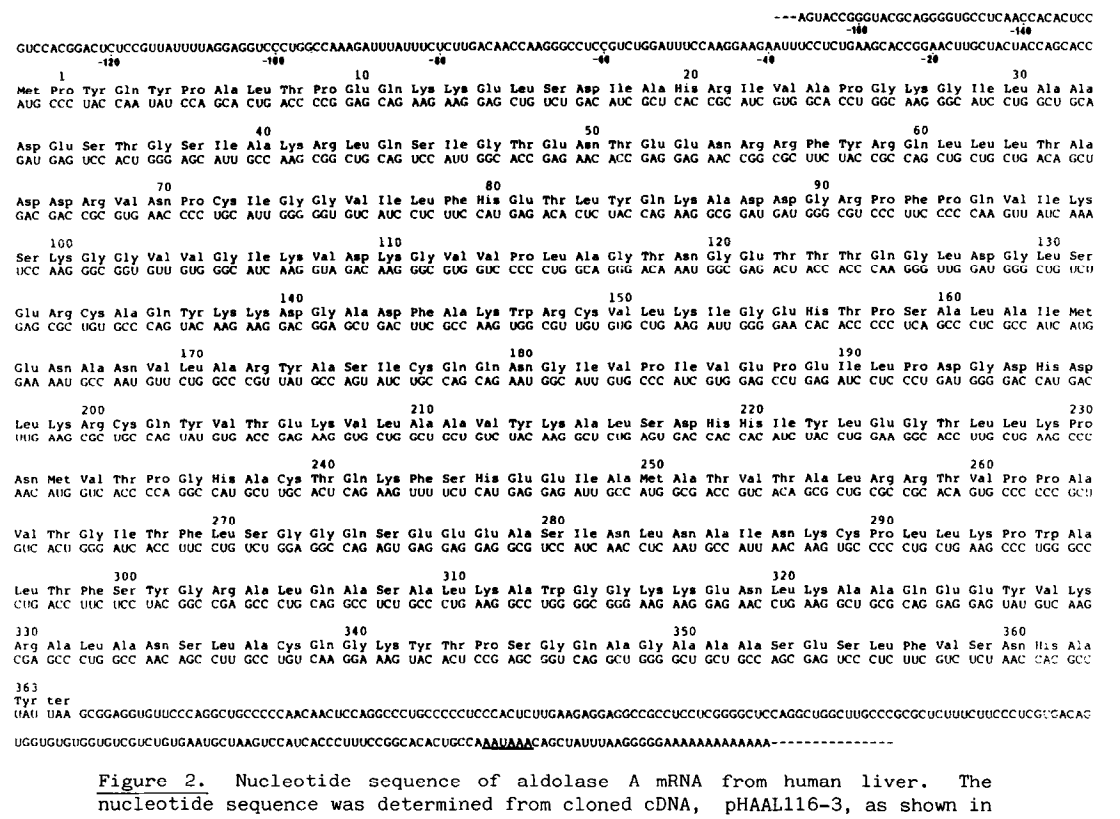

Figure 2. Nucleotide sequence of aldolase A mRNA from human liver. The nucleotide sequence was determined from cloned cDNA, pHAAL116-3, as shown in Figure 1. The amino acid sequence shown is deduced from the possible open reading frame. Polyadenylation signal in 3'-noncoding region is underlined.

region (Figure 3); the 5'-noncoding stretch of human aldolase A mRNA is almost twice as long as the other mRNAs and the far upstream nucleotide sequence is also quite different from that of rat and rabbit mRNAs both of which are quite similar to one another. The clone, pHAAL116-3, is most likely to be a genuine sequence which can not be attributed to a cloning artifact,

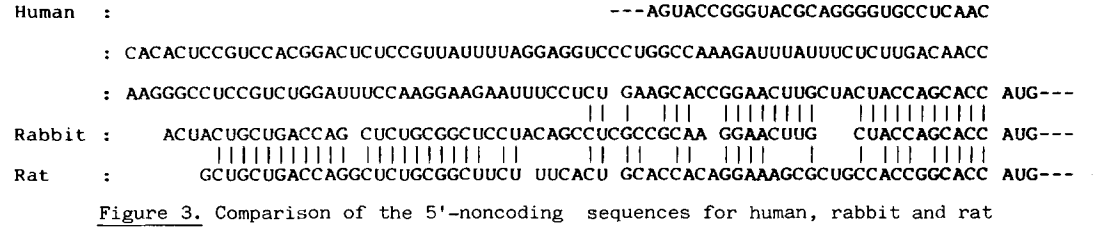

Figure 3. Comparison of the 5'-noncoding sequences for human, rabbit and rat aldolase A mRNAs. 5'-noncoding region is completely sequenced with the rabbit mRNA but approximately 40 nucleotides for the human mRNA and a few nucleotides for the rat mRNA remain to be determined. In aligning three sequences, gaps were introduced to maximize the homology. Bars between the two sequences indicate the identical bases in between. Sequence of a rabbit aldolase A mRNA was determined by Tolan et al.(12). Sequence of a rat aldolase A mRNA was determined by us (4).

because the cleavage map of the corresponding region was identical with other cDNA clone isolated independently. Furthermore a pseudogene which we isolated through a genomic cloning and turned out to be a "processed gene" carrying the same DNA sequence with that of pHAAL116-3 (data not shown).

Different aldolase A mRNAs: RNA blotting has been done to determine the molecular size of aldolase A mRNA isolated from various tissues. Unexpectedly, at least two molecular species of aldolase A mRNA were detected, even though a specific probe was used to avoid the cross-hybridization with aldolase B mRNA (Figure 4). Their sizes were estimated to be 1,600 nucleotides for a skeletal muscle mRNA and 1,700 nucleotides for the liver and placenta mRNAs. The difference of around 100 nucleotides in length between the mRNAs persisted even after removal of the poly(A) tail (data not shown), indicating that these aldolase A mRNA species with a different chain length were expressed in tissue-specific manner.

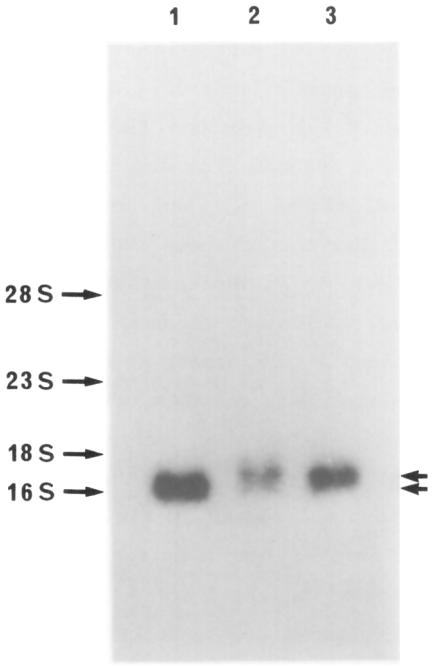

Figure 4. RNA blotting of poly(A)$^+$ RNAs from various human tissues. RNA blotting was performed as described in Materials and Methods. Poly(A)$^+$ RNAs are from skeletal muscle ((1), 1 µg), adult liver ((2), 10µg) and placenta ((3), 5µg). The arrows at right hand side indicate the position of the human aldolase A mRNA, whose molecular size was estimated to be 1,600 nucleotides for the skeletal muscle mRNA and 1,700 nucleotides for the liver and placenta mRNAs. Repeated experiments gave the same results. Molecular weight makers are 16S, 18S, 23S and 28S rRNAs.

DISCUSSION

We have isolated several cDNA clones that encode human aldolase A by screening a human liver cDNA library. One of cDNA clones, pHAAL116-3, is composed of 1,464 nucleotides, excluding poly(A) tail and seems to cover more than 95% of the entire sequence of aldolase A mRNA considering the size of the liver mRNA and its poly(A) tail, that is, pHAAL116-3 contained a nearly complete 5'-noncoding sequence, the entire coding sequence and the complete 3'-noncoding sequence. The coding region was identified by comparing the sequence with the corresponding sequences of rabbit (12) and rat (4) skeletal muscle aldolase A cDNAs and partial amino acid sequence of human aldolase A (22). A comparison of nucleotide sequences of the human aldolase A mRNA with the rabbit and rat skeletal muscle aldolase A mRNAs shows that the degree of homology is high in their coding and 3'-noncoding regions, but is considerably lower in the 5'-noncoding region, especially at the upstream region (Figure 3), although the high sequence homology remains between these rodent mRNAs.

Different species of aldolase A mRNAs with distinct size were detected in different tissues. The length of the mRNA being expressed in skeletal muscle was 1,600 nucleotides, while that from the liver and placenta tissues was 1,700 nucleotides. The results suggest that at least two mRNAs differing in length are under tissue-specific control. Since only a single molecular species of human aldolase A was detected (22) and number of amino acid residues of human aldolase A deduced from the cDNA sequences is the same to the rabbit (12) and rat aldolases A, the coding region seems not to be responsible for the size difference of these mRNAs. A similar result was also observed in the rat (24, 25). In contrast to the high sequence homology of the 3'-noncoding regions, nucleotide sequence and size of the 5'-noncoding region of human liver aldolase A mRNA appear to be quite different from those of the rabbit and rat skeletal muscle mRNAs (Figure 3). The difference in length of the mRNA was approximately 100 nucleotides-long between human and rodent A mRNAs as observed in Fig.3. Since these differences correspond to the difference of the 5'-terminal region in Fig.4, it is most likely that this region is responsible for the length heterogeneity of the mRNA, assuming that the both human and rodent muscle A mRNAs possess the same size of the 5'-noncoding region. As to the sequence heterogeneity the human liver mRNA certainly hybridized to a DNA fragment at the 5'-end of pHAAL116-3 clone, but did not hybridize with a 5'-terminal fragment of a rat skeletal muscle aldolase A cDNA even at a low stringency in RNA blotting. However, poly(A)$^+$ RNA from human skeletal muscle did hybridize with the same 5'-terminal fragment of a rat skeletal muscle aldolase A cDNA whose size corresponded to that of the human skeletal muscle aldolase A mRNA (data not shown). These

results suggest that the sequence at 5'-end of human skeletal muscle aldolase A mRNA is substantially homologous to that of the rat skeletal muscle aldolase A mRNA but differs from a 5'-extreme terminus of the human liver mRNA. It is, therefore, probable that the discrepancy seen in the 5'-terminal structure between the human liver mRNA and two rodent skeletal muscle mRNAs is also true for the case of human liver and skeletal muscle mRNAs. This is not due to the species-specificity, but rather due to tissue-specific expression. Further analyses of the nucleotide sequence of human aldolase A mRNA from the skeletal muscle, especially that of the 5'-terminal region which may only differ is needed to prove the possibility. There are a number of examples of sequence difference at the 5'-end among mRNAs from a single gene, such as α-amylase gene (26), Drosophila alcohol dehydrogenase gene (27) and others (28,29). It is not determined yet at present whether or not these mRNAs are transcribed from a single gene. It is interesting to clarify the structural relationship of these mRNAs to a chromosomal gene.

Aldolase A is found to be expressed in almost all the tissues of animal. In rat skeletal muscle it represents as much as 5% of total cellular proteins (30). On the contrary, in an adult liver aldolase B is preferentially expressed but aldolase A is only partially expressed (21). Similar results also have been observed at a transcriptional level (24, 31). In fact we have obtained aldolase A cDNA clones at a frequency of 0.01% of total transformants, whereas aldolase B cDNA clones were obtained from the same library at more than ten times the frequency of aldolase A cDNA clones (23). The proportion of aldolase A mRNA in the liver is estimated to be less than one-tenth from the intensities of the band compared to that in the skeletal muscle (Figure 4) considering the amounts of the poly(A)$^+$ RNA applied in the lane. The evidences presented in this paper indicate that, as the skeletal muscle exclusively expresses the shorter mRNA but not the longer one, it was essential for the cloning of the longer one from the liver although the isolation of aldolase A cDNA clones from the liver was, at the beginning, thought to be relatively difficult.

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