Isozyme-specific Modules on Human Aldolase A Molecule

ISOZYME GROUP-SPECIFIC SEQUENCES 1 AND 4 ARE REQUIRED FOR SHOWING CHARACTERISTICS AS ALDOLASE A*

(Received for publication, July 28, 1992)

Kiyohisa Motoki, Yoshihiko Kitajima, and Katsuji Hori‡

From the Department of Biochemistry, Saga Medical School, Nabeshima, Saga 849, Japan

Vertebrate aldolase molecules bear at least four stretches of isozyme group-specific sequences (referred to as IGS). The IGSs of the type A isozyme are known to endow the aldolase molecules with some characteristics typical of A. In order to locate the type A regions, 4 chimeric enzymes were constructed between human aldolases A and B and 5 mutant enzymes with single or double mutations in the IGS-1 region. Among engineered proteins, the chimeric enzymes bearing the type A IGS-1 to -4 (BAB34-108:306-363) and the IGS-1 and -4 (BAB34-55:306-363) exhibited similarities to isozyme A in many respects. On the other hand, neither chimeric enzyme bearing the type A IGS-1 to -3 (BAB34-108) nor that bearing the IGS-1 alone (BAB34-55) exhibited properties as isozyme A. Four mutant aldolases A (carrying single mutation in the IGS-1 region) maintained the original activity as A. Similarly, the BA306 chimera with the type B → A substitution at positions 41 and 45 (BA306 N41K.R45S) failed to exhibit the A-like properties although the activities toward Fru-1,6-P₂ and Fru-1-P significantly increased. Conclusively, the type A IGS-1, together with the IGS-4, act as indispensable modules in determining the characteristic properties of human aldolase A.

A, B, and C are three isozymic forms in vertebrate aldolase (EC 1.4.2.13). The three play key roles in glycolysis (1). Aldolase A, the muscle type isozyme, has a preference for Fru-1,6-P₂ rather than Fru-1-P because, primarily, the isozyme is responsible for a generation of chemical energy in the skeletal and cardiac muscles and other tissues, and, secondly, these tissues in which aldolase A works lack the biochemical pathway to metabolize fructose to glycolytic intermediates via the Fru-1-P production. On the contrary, aldolase B, the liver-type isozyme, offers the substrate specificity suitable for the metabolism in the hepatocytes where fructose is metabolized to pyruvate via Fru-1-P; that is, the same catalytic activities toward both Fru-1,6-P₂ and Fru-1-P. Similarly, aldolase C, the brain-type isozyme, functions in fetal tissues and adult nerve tissues and therefore shows an intermediate activity toward Fru-1,6-P₂/Fru-1-P with the ratio of approximately 10:1.2

Recently, x-ray crystallographic studies indicated that muscle-type aldolases of rabbit (3) and humans (4, 5) have a pseudo-8-fold β/α-barrel structure. The core structures of Drosophila aldolase have also been shown to be essentially identical with those of the vertebrate aldolases (6, 7). Comparison of amino acid sequences of vertebrate aldolase isozymes reveals that the enzyme molecules are composed of several short and long stretches of amino acid sequences belonging to three different categories: (a) the sequences commonly conserved among three isozymic groups (referred to as CCS), (b) isozyme group-specific sequences (referred to as IGS), and (c) divergent sequences (8). The CCSs are the major composition of the β/α repeating structures and their connecting turns that form the 8-fold β/α-barrel (8). The IGSs are located at three sites in the amino-terminal regions (IGS-1, -2, and -3) and in the carboxyl termini (IGS-4) of the enzymes (8). These molecular features of aldolases made the determination of a possible role of the IGSs in connection with an isozyme group-specific function of the enzyme very tempting. Thus, chimeric enzymes were systematically constructed between human aldolases A and B, and their characteristics were analyzed (8). Previous studies have shown that for aldolase A, the carboxyl-terminal region bearing the Tyr-363 (IGS-4) and the amino-terminal region spanning amino acid residues 34–108 (IGS-1–3) serve as the determinants that exhibit characteristics of isozyme A (8). The significance of Tyr-363 and the proximate carboxyl-terminal region in determining the characteristics of isozyme A have been elucidated by several different studies using the chimeric enzyme construction (8), the enzymatic modifications (9, 10), and the site-directed mutagenesis (11, 12). The importance of the carboxyl termini on isoamylase-specific catalysis can be drawn from the fact that Drosophila aldolase has three isozyme forms with distinct carboxyl-terminal sequences corresponding to the IGS-4 of vertebrate aldolase (13–15).2

In order to precisely locate the regions or residues necessary for the determination of isozyme specificity, various types of chimeric enzymes were constructed between human aldolases A and B and also the mutant aldolases (with single or double mutations in the IGS-1 region). The enzymatic properties of these constructs were later analyzed. This communication postulates that the type A IGS-1, together with the IGS-4, act as indispensable modules in determining the characteristic properties of aldolase A.

* This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence and reprint requests should be addressed.

1 The abbreviations used are: Fru-1,6-P₂, fructose 1,6-bisphosphate; Fru-1-P, fructose 1-phosphate; CCS, conserved common sequence; IGS, isozyme group-specific sequence; PAGE, polyacrylamide gel electrophoresis; mAb, monoclonal antibody.

2 R. Zhang, T. Kai, Y. Sugimoto, Y. Takasaki, K. Kaga, and K. Hori, unpublished data.
**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Plasmids—*Escherichia coli* K12 strain JM83 from laboratory stock was used for the cloning and expression experiments. pINIII (16), an *E. coli* expression vector that contains lipoprotein promoter (Ipp), lac UV5 promoter operator (lac), and lactose repressor gene (lacI), were as previously described (17). pHAA116-3, a cDNA clone of human aldolase A, and pHABL120-3, a cDNA clone of human aldolase B, both of which were isolated in the laboratory as previously described (17), were used to construct the expression plasmids.

**Enzymes and Other Materials—Restriction enzymes, T4 DNA ligase, T4 polynucleotide kinase, and other enzymes were purchased from Takara Biochemicals, Nippon Gene and Toyobo Co., Ltd. Mu- tagenesis kit. 

**Construction and Expression of Plasmids for Chimeric Enzymes—** cDNAs for chimeric enzymes were constructed by connecting cDNA fragments for isozymes A and B (11) and that for chimeric enzyme BA34 (Fig. 1I) (8). pHAA-A47, an expression plasmid of human aldolase A, pHA-B141, an expression plasmid of human aldolase B, and pHA-BA34, an expression plasmid of chimeric enzyme BA34, were digested with restriction endonucleases at various sites common to pH-BA34, an expression plasmid of chimeric enzyme BA34, were constructed as described in a previous paper (17).

**Construction Diagrams of the Expression Plasmids**—Restriction maps of human aldolase A, B, and BA34 cDNAs (Fig. 1I) (8). pHAA-A47, an expression plasmid of human aldolase A, pHA-B141, an expression plasmid of human aldolase B, and pHA-BA34, an expression plasmid of chimeric enzyme BA34, were digested with restriction endonucleases at various sites common to pH-BA34, an expression plasmid of chimeric enzyme BA34, were constructed as described in a previous paper (17).

**Isozyme Group-specific Modules**—The noncoding regions of the expression plasmids are represented by open boxes. Arrows in expression plasmids represent *E. coli* protein promoter (Ipp). a, lac UV5 promoter operator (lac), and lactose repressor gene (lacI), were as previously described (17). pHAA116-3, a cDNA clone of human aldolase A, and pHABL120-3, a cDNA clone of human aldolase B, both of which were isolated in the laboratory as previously described (17), were used to construct the expression plasmids.

**Fig. 1.** Construction of *E. coli* expression plasmids harboring cDNA encoding human aldolase A and B chimeric proteins between isozymes A and B. **I.** Construction diagrams of the expression plasmids for two BAB chimeric enzymes; **II.** Construction diagrams of the expression plasmid for the BABA chimeric enzymes. The coding regions of isozyme A and B expression plasmids and the corresponding amino acid sequences are represented by stippled and closed boxes, respectively. The noncoding regions of the expression plasmids are represented by open boxes. Arrows in expression plasmids represent *E. coli* lipoprotein promoter (Ipp) and lactose (lac) promoters derived from the pINIII vector as shown in a previous paper (17). Thin lines in expression plasmids were carried out by using the Amersham vector Ac, Accl; Pg, BglII; Cfr, CfrI; H, HindIII; Hf, HindI, pHA-A47, an *E. coli* expression plasmid for isozyme A, pHA-B141, an expression plasmid for isozyme B, and pHA-BA34, an expression plasmid for chimeric isozyme BA34, were constructed as described in a previous paper (17).
tometric methods. In the staining method, 10 μl each of enzyme preparations was applied to cellulose polyacetate strips, subjected to electrophoresis for 40 min at 250 V at 4 °C, and stained for aldolase activity in the presence of 5 mM EDTA (to inhibit E. coli class II aldolase activity) (20). The spectrophotometric assay was performed at 30 °C according to Rajkumar et al. (21).

Determination of Molecular Sizes—The molecular sizes of the wild type and the engineered aldolases were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (22). Those of the native tetrameric forms of aldolases were determined as previously described (23).

Immunoblotting—Immunoblotting was carried out as previously described (24).

Oligonucleotide-directed, Site-specific Mutagenesis—Mutagenesis was carried out by using the Amersham oligonucleotide-directed in vitro mutagenesis kit (25–27). The CDNA inserts of human aldolase A (pHA-A47) and BA306 (pHA-BA306) in E. coli expression plasmids were cloned into the M13mp9 phage to synthesize the template for mutagenesis. The mutagenic oligonucleotides employed in this study and the corresponding amino acid substitutions are listed in Table I. Screening of positive plaques for mutants was performed directly by dideoxy DNA sequencing (18). The mutant enzymes of aldolase A bearing a single replacement, Lys-41 → Arg, Lys-41 → Asn, Arg-42 → Lys, Ser-45 → Arg, are referred to as A-K41R, A-K41N, A-R42K, and A-S45R, respectively. The mutant enzyme of BA306 carrying double mutations, Asn-41 → Lys and Arg-45 → Ser, is referred to as BA306N41K,R45S.

RESULTS AND DISCUSSION

Comparison of Primary Structures of Vertebrate Aldolases

There were at least 7 highly conserved sequences (CCS) and 4 isozyme group-specific sequences (IGS) in the primary structure of the vertebrate aldolases from various sources (Fig. 2 and Ref. 8). The CCSs appeared to be the modules for the basal framework of β/α-barrel structure and are required to exhibit the activity common to the three isozymic forms of vertebrate aldolase (8). In particular, CCS-5 is the longest stretch bearing Lys-229 as the active site of the enzyme and Glu-187/189 as the presumptive C-1-P binding residue (3, 5), each of which is highly conserved through aldolases A, B, and C. By contrast, the IGSs were conserved within a single isozyme group or two. As shown in Fig. 2, IGSs are the modules where amino acid substitutions occurred in an isozyme group-specific manner and basically overlap the additional α-helices which could be inserted into or duplicated locally by a regular repetition of β/α-structures to give three α/α helices, A1/A2, B1/B2, and H1/H2 (3) of aldolase molecule when evolved.

Analyses with Chimeric Molecules of Human Aldolases A and B

Construction of Chimeric Enzymes between Human Aldolases A and B

To ascertain whether these IGSs really determine isozyme-specific functions, E. coli expression plasmids were constructed for four chimeric enzymes between isozymes A and B (Fig. 1). In this study, local sequences of isozyme A were introduced but restricted to IGS-1, IGS-1 through -3, and IGS-4 with four different combinations into the respective regions of isozyme B. The two BAB chimeric enzymes, BAB34-55 and BAB34-108, carried the amino acid sequences with either the type A IGS-1 (amino acid residues 34–55) or IGS-1–3 (amino acid residues 34–108) between the amino-terminal and the carboxyl-terminal fragments of isozyme B. The two other chimeric enzymes, BAB34–55:306–363 and BAB34–108:306–363, were the derivatives of BAB34–55 and BAB34–108, respectively, in which the type A IGS-4 (the amino acid residues 306–363) was substituted for that of the type B isozyme (Fig. 1).

Expression of Chimeric Enzymes

These constructions were transfected into E. coli JM83 as described under “Experimental Procedures.” Cell extracts of E. coli transfected with the expression plasmids were assayed for aldolase activity using the activity staining method (20). BAB34-108 did not show activity at all. BAB34-55, BAB34–108:306–363, and BA306 as controls gave detectable activity spots, while BAB34–55:306–363 was as strong as aldolase A (Fig. 3). The generated enzymes were purified by homogeneity as previously described (11). These chimeric proteins moved toward the anode with electrophoretic mobilities predicted from their isoelectric points.

Table I

| Amino acid | Wild-type human aldolase A and BA306 genes and mutant oligonucleotide sequence | Substitution amino acid | Plasmid       |
|------------|--------------------------------------------------------------------------------|------------------------|---------------|
| Wild-type A template | 41 42 | Wild-type human aldolase A and BA306 genes and mutant oligonucleotide sequence | Lys-41 → Arg | pHA-A47 K41R |
| Mutagenic oligonucleotide | 45 | Wild-type human aldolase A and BA306 genes and mutant oligonucleotide sequence | Lys-41 → Arg | pHA-A47 K41R |
| 1 | 5'-C ATT GCC AGG CGG CTG CA-3' | Wild-type human aldolase A and BA306 genes and mutant oligonucleotide sequence | Lys-41 → Arg | pHA-A47 K41R |
| 2 | 5'-C ATT GCC AAC CGG CTG CA-3' | Wild-type human aldolase A and BA306 genes and mutant oligonucleotide sequence | Lys-41 → Arg | pHA-A47 K41R |
| 3 | 5'-ATT GCC AAG AGG CTG CAG TTG-3' | Wild-type human aldolase A and BA306 genes and mutant oligonucleotide sequence | Lys-41 → Arg | pHA-A47 K41R |
| 4 | 5'-CGG CTG CAG GGC ATT GGC ACC-3' | Wild-type human aldolase A and BA306 genes and mutant oligonucleotide sequence | Lys-41 → Arg | pHA-A47 K41R |
| Amino acid | BA306 template | Wild-type human aldolase A and BA306 genes and mutant oligonucleotide sequence | Lys-41 → Arg | pHA-A47 K41R |
| Mutagenic oligonucleotide | 45 | Wild-type human aldolase A and BA306 genes and mutant oligonucleotide sequence | Lys-41 → Arg | pHA-A47 K41R |
| 5 | 5'-ATG GGG AGG CGG CTG CAG-3' | Wild-type human aldolase A and BA306 genes and mutant oligonucleotide sequence | Lys-41 → Arg | pHA-A47 K41R |
| 6 | 5'-CTG CAG AGC ATG TAG CGT-3' | Wild-type human aldolase A and BA306 genes and mutant oligonucleotide sequence | Lys-41 → Arg | pHA-A47 K41R |
I.

11. IGS-1 and &strands are shown with large and aldolase aldolases they have almost the same molecular sizes (8). When the were carried out under the conditions described under "Experimental previous study, it was shown that human aldolases A and R purified enzymes were subjected to a SDS-PAGE, the BAR moved with different mobilities in a SDS-PAGE Characteristics

II.

Fig. 2 Schematic presentation of human aldolase isozymes A and B. I. the structures of human aldolases A and B are depicted. The name and position of the functional amino acid residues are indicated at the top of A and at the bottom of B, respectively. The α-helices and β-strands are shown with large and small Roman characters above the corresponding horizontal lines. The characteristic sequences (CCS-1, 7 and IGS-1-4) are shown with open and closed bars with their names. II. The amino acid sequences in the four IGS regions of vertebrate aldolases A, B, and C are aligned. Asterisks represent the positions where amino acid residues are deleted. Literature sources are as follows: aldolase A from humans (28), rabbit (29), rat (30), aldolase B from human (31), rat (32), and chicken (33); aldolase C from humans (34), rat (35), and chicken (36).

Fig. 3 Zymograms of normal and chimeric human aldolases expressed in E. coli. Electrophoresis and activity staining were carried out under the conditions described under "Experimental Procedures." Activity spots in front of those for human aldolase A and B and their chimeric enzymes moving toward the anode are of E. coli class II aldolase. JM83, untransfected E. coli extract as control. O, electrophoresis origin; + and −, the anodic and cathodic sides, respectively.

Characteristics of Chimeric Enzymes

Electrophoretic Mobilities of Chimeric Enzymes—In a previous study, it was shown that human aldolases A and B moved with different mobilities in a SDS-PAGE as if the latter was apparently much smaller than the former although they have almost the same molecular sizes (8). When the purified enzymes were subjected to a SDS-PAGE, the BAB chimeric enzymes resembled aldolase B in their electrophoretic mobilities, while the BABA chimeras rather resembled aldolase A in their mobilities (data not shown).

Reactivity of These Enzymes with Monoclonal Antibodies, mAbB1A2 and mAbB4C2—To ascertain if these chimeric enzymes were constructed with a structure as expected, an immunoblotting test was run by using monoclonal antibodies for human aldolase A, mAbB1A2 and -4C2. mAbB1A2 reacts with the carboxyl-terminal region of the enzyme spanning amino acid residues 306-363, while mAbB4C2 recognizes an epitope at the amino-terminal region of the enzyme present within amino acid residues 34-108 (24). As shown in Fig. 4, the two BABA chimeric enzymes as well as aldolase A reacted with mAbB1A2, whereas the two BAB chimeric enzymes which lacked the type A carboxyl-terminal sequences and the human aldolase B did not react with the antibody. Similarly, chimeric enzymes carrying the type A sequences from 34 to 108 (BABA34-108, BABA34-108:306-363) interacted with mAbB4C2 as expected, whereas chimera with the type A sequences from 34 to 55 (BABA34-55 and BABA34-55:306-363) escaped from an interaction with the antibody. These results indicate that the chimeric enzymes employed in this study were constructed as expected and, furthermore, showed that the epitope recognizable by mAbB4C2 should be on the amino acid sequences from 56 to 108 of the type A isozyme.

General Properties of Chimeric Enzymes

In this study, the same criteria as those used in a previous work to discriminate the type A and B chimeric enzymes from
Acid mAb4C2 recognized an epitope human aldolase A. mAb1A2 reacted with sites located within amino acid residues 55-108 at the amino-terminal region.

**TABLE II**

**Characteristics of normal and chimeric forms of human aldolase**

The $K_{m}$ values of normal and chimeric enzymes for Fru-1,6-P$_2$ and Fru-1-P were determined by a Lineweaver-Burk plot with data determined by measuring activities in the presence of various amounts of substrates under standard assay conditions (22).

| Enzyme | $K_{m}$ Fru-1-P | Activity ratio Fru-1-P/Fru-1,6-P$_2$ | $K_{m}$ Fru-1,6-P$_2$ |
|--------|----------------|----------------------------------|-------------------|
|        | (min$^{-1}$)   |                                  | (m$M^{-1}$)       |
| M     | A              | B                                | M                |
|       | 2768           | 48                               | 58               |
|       | $2.7 	imes 10^{-5}$ | $2.7 	imes 10^{-2}$               | $2.7 	imes 10^{-2}$ |
|       | 256            | 224                              | 1.1              |
|       | $1.2 	imes 10^{-5}$ | $3.7 	imes 10^{-3}$               | $3.7 	imes 10^{-3}$ |
|       | 560            | 384                              | 1.5              |
|       | $7.4 	imes 10^{-6}$ | $2.6 	imes 10^{-3}$               | $2.6 	imes 10^{-3}$ |
| ABA34-108 | <16 <16 ND$^a$  | ND$^a$                            | ND$^a$           |
|        | 688            | 96                               | 7.2              |
|        | $2.7 	imes 10^{-5}$ | $3.5 	imes 10^{-3}$               | $3.5 	imes 10^{-3}$ |
| BABA34-55 | 128 80       | 1.6                              | $8.3 	imes 10^{-5}$ |
|        | $4.2 	imes 10^{-3}$ | $1.7 	imes 10^{-2}$               | $1.7 	imes 10^{-2}$ |
| BABA34-55 | 1120 64      | 18                               | $4.8 	imes 10^{-5}$ |

$^a$ND is not determined because of a rapid loss of activity.

The results strongly suggest that the type A IGS-1 and -4 are mainly responsible for exhibiting characteristics as the isozyme A and also indicate that IGS-2 and -3 are not likely to play a role in the functions directly related to the activity toward Fru-1,6-P$_2$ and Fru-1-P. In a previous paper (8), it was shown that an internal region spanning positions 108-212 would be responsible for regulating the catalytic activities toward Fru-1-P; low for aldolase A and high for aldolase B (8). This was presumably because the region carried amino acid residues implicated to be the C-1-phosphate binding sites (3, 5); moreover, BABA108 chimera exhibited a low catalytic activity toward Fru-1-P like aldolase A, whereas BABA12 showed a high Fru-1-P activity like aldolase B (8). However, the present results show definitely that the type A IGS-1, in conjunction with IGS-4, modulate the catalytic activity of aldolase A toward Fru-1,6-P$_2$ at a high level and that toward Fru-1-P at a low level. This indicates that the IGS-1 would be superior to the internal sequences covering positions 108 to 212 in determining the enzyme activity toward Fru-1-P. It is also likely that the type B IGS-2 and -3 would be responsible for the conformational stability of aldolase B since BABA34-55 was more stable than BABA34-108. General properties of these chimeric proteins are summarized in Table II.

**Analyses with Aldolases Bearing a Single or Double Mutation in IGS-1**

The results obtained with four chimeric enzymes constructed in this study strongly suggest that IGS-1 play a crucial role in exhibiting the properties of the type A isozyme. As can be seen in Fig. 2B, 8 out of 13 amino acid residues in the IGS-1 of human aldolases A and B are different from one another. Therefore, it is of particular interest to ascertain which residue(s) in IGS-1 play the role of determining the
Function of Isozyme Group-specific Modules of Aldolase A

Specificity toward Fru-1,6-P$_2$ and Fru-1-P. The type A IGS-1 bears Lys-41 and Arg-42, both of which have been implicated to be the C-6-phosphate binding sites from X-ray crystallographic studies (5), and Ser-45, a residue also implicated to be of importance in determining the specificity toward Fru-1,6-P$_2$ and Fru-1-P (6). Thus, four mutant enzymes of isozyme A were constructed, each of which had single mutations at the respective sites, to examine whether the residues in the parent enzyme were really indispensable for the activity (Table III). The A-K41R and the A-K41N were derivatives of human aldolase A in which Lys-41 was replaced by Arg, a positively charged residue, in the former and by the type B residue, Asn, in the latter, respectively. The A-R42K is a derivative of the isozyme A carrying the Arg-42 → Lys substitution. Similarly, the A-S45R is a derivative of isozyme A with the Ser-45 → Arg substitution. All derivatives of isozyme A were active and maintained almost the same activities toward Fru-1,6-P$_2$ and Fru-1-P as that of the parent isozyme A (Table III). Thus, it appears that amino acid residues at positions 41 and 42 are replaceable with another residue having a similar property. The Ser-45 of aldolase A is likely to be dispensable since the mutant with the Ser-45 → Arg substitution was still active (Table III). BA306 N41K/R45S is a derivative of the chimeric enzyme BA306 carrying the double mutations at positions 41 and 45 as the Asn-41 → Lys and the Arg-45 → Ser substitutions. The effects of the double mutations on the catalytic activities toward Fru-1,6-P$_2$ and Fru-1-P were prominent, and the $k_{cat}$ values for these substrates were likely to be 1.6-fold as compared to those of BA306 (Table III), indicating that the type A residues, Lys-41 and Ser-45, play together a role of conferring isozyme-specific catalysis. Therefore, the type A IGS per se could be required as a module for exhibiting the activity like isozyme A, although there is another possibility that amino acid residues other than Lys-41 and Ser-45 play a role in the process. Although the data presented here lead to the conclusion that IGS-1, together with IGS-4, would be particularly important in conferring isozyme-specific catalysis of the particular isozyme, more evidences are needed to show which residues or what structure is really responsible for the activity. At present, the functions of IGS-2 and -3 remain unknown. Since the amino acid sequences covering the IGS-2 and -3 are situated at the surface opposite to the region where the subunit contact occurs (3-5), the IGS-2 and -3 regions might serve as the isozyme-specific modules for conformational stabilization, the tissue-specific interaction with other glycolytic enzymes, or cytoskeletal proteins in cellular compartments (37, 38).

The results obtained with several chimeric enzymes and the mutant enzymes with single or double mutations clearly indicate that the isozyme group-specific sequences in human aldolases A and B would play a positive role in elucidating isozymic functions such as a high or low catalytic activity toward Fru-1,6-P$_2$ and Fru-1-P, the $K_M$ for these substrates and so on. Therefore, it may be speculated that the three isozymic forms of vertebrate aldolase might be generated from an ancestor through multiple mutations accumulated mainly in the IGS regions which can be mapped to the A1/A2, B1/B2, and H1/H2 sites, the $\alpha/\alpha$-helices located at the flanking region of the regular 8-fold $\beta/\alpha$-barrel structure.  

Acknowledgments—We thank Professor Hiromu Kohda for interest and encouragement during this work. We also thank Drs. Yozo K. Hori, K. Motoki, and Y. Kitajima, manuscript in preparation.

3 K. Hori, K. Motoki, and Y. Kitajima, manuscript in preparation.
Takasaki, Mamoru Oh-uchida, and Tatsuo Kai for helpful discussions and Hitomi Yatsuki for technical assistance.

REFERENCES

1. Horecker, B. L., Tolan, O., and Lai, C. Y. (1972) in The Enzymes (Boyer, P. D., ed) pp. 213-258, Academic Press, New York
2. Leberherz, H. G., and Rutter, W. J. (1973) J. Biol. Chem. 248, 1650-1659
3. Sypniew, J., Benardis, Y., and Allaire, M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7845-7850
4. Gambin, S. J., Cooper, B., Millar, J. R., Davis, G. J., Littlechild, J. A., and Watson, H. C. (1990) FEBS Lett. 262, 292-296
5. Gambin, S. J., Davies, G. J., Grims, J. M., Jackson, R. M., Littlechild, J. A., and Watson, H. C. (1991) J. Mol. Biol. 219, 573-576
6. Brenner-Holzach, O., and Smit, J. D. G. (1982) J. Biol. Chem. 257, 11747-11749
7. Hester, G., Brenner-Holzach, O., Rossi, F. A., Struck, Donats, M., Winterhalter, K. H., Smit, J. D. G., and Piennke, K. (1991) FEBS Lett. 292, 237-242
8. Kitajima, Y., Takasaki, Y., Takahashi, I., and Hori, K. (1990) J. Biol. Chem. 265, 17483-17488
9. Drechsler, E. R., Boyer, P. D., and Kowalsky, A. G. (1959) J. Biol. Chem. 234, 2627-2634
10. Rutter, W. J., Richards, O. C., and Woodfin, B. M. (1961) J. Biol. Chem. 236, 3193-3197
11. Takahashi, I., Takasaki, Y., and Hori, K. (1989) J. Biochem. (Tokyo) 105, 291-296
12. Takasaki, Y., Takahashi, I., Mukai, T., and Hori, K. (1990) Proc. Clin. Biol. Res. 344, 935-955
13. Shaw-Lee, R., Lissimore, J. L., Sullivan, D. T., and Tolan, D. R. (1992) J. Biol. Chem. 267, 3969-3976
14. Kim, J., Yun, J. J., Wang, S., and Dorsett, D. (1992) Mol. Cell. Biol. 12, 773-783
15. Kai, T., Sugimoto, Y., Kusakabe, T., Zhang, R., Koga, K., and Hori, K. (1992) J. Biochem. (Tokyo) 112, 677-688
16. Nakamura, K., and Inouye, M. (1982) EMBO J. 1, 771-775
17. Sakakibara, M., Takahashi, I., Takasaki, Y., Mukai, T., and Hori, K. (1989) Biochim. Biophys. Acts 1007, 334-342
18. Messing, J. (1983) Methods Enzymol. 101, 20-78
19. Penhoet, E. E., Rajkumar, T., and Rutter, W. J. (1966) Proc. Natl. Acad. Sci. U. S. A. 56, 1275-1262
20. Susan, W. A., Penhoet, E. E., and Rutter, W. J. (1975) Methods Enzymol. 41, 66-73
21. Rajkumar, T. V., Woodfin, B. M., and Rutter, W. J. (1966) Methods Enzymol. 9, 491-498
22. Lasenby, U. K. (1970) Nature 227, 660-668
23. Martin, R. G., and Arkes, B. N. (1981) J. Biol. Chem. 256, 1372-1379
24. Kitajima, Y., Matsubashi, S., Nishida, H., Takasaki, Y., Takahashi, I., Hotta, T., and Hori, K. (1990) J. Biochem. (Tokyo) 109, 544-550
25. Taylor, J. W., Schmidt, W., Coast, R., Okruszek, A., and Eckstein, F. (1980) Nucleic Acids Res. 13, 8749-8764
26. Taylor, J. W., Ott, J., and Eckstein, F. (1985) Nucleic Acids Res. 13, 8764-8785
27. Nakayama, K., and Eckstein, F. (1986) Nucleic Acids Res. 14, 9679-9698
28. Sakakibara, M., Mukai, T., and Hori, K. (1985) Biochem. Biophys. Res. Commun. 131, 413-420
29. Tolan, D. R., Amsden, A. B., Putney, S. D., Ordea, M. S., and Penhoet, E. E. (1984) J. Biol. Chem. 259, 1127-1131
30. Joh, K., Mukai, T., Yatsuki, H., and Hori, K. (1995) Gene (Amst.) 39, 17-24
31. Sakakibara, M., Mukai, T., Yatsuki, H., and Hori, K. (1985) Nucleic Acids Res. 13, 5050-5069
32. Tatsumi, K., Mukai, T., Tsutsumi, R., Hidaka, S., Arai, Y., Hori, K., and Ishikawa, K. (1985) J. Mol. Biol. 181, 153-160
33. Rottmann, W. H., Deselms, K. R., Niclas J. Camerato, T., Holman, P. S., and Klung, T. (1987) Biochimie (Paris) 69, 137-145
34. Kukita, A., Mukai, T., Miyata, T., and Hori, K. (1988) Eur. J. Biochem. 171, 471-478
35. Ono, K., Tatsumi, K., and Ishikawa, K. (1990) Biochem. Int. 20, 921-929
36. Masters, C. (1984) J. Cell Biol. 98, 225a-225a
37. Clarke, P., Stephan, P., Morton, D., and Weidemann, J. (1985) in Regulation of Carbohydrate Metabolism (Beitner, R., ed) Vol. II, pp. 1-31, CRC Press, Boca Raton, FL

Function of Isozyme Group-specific Modules of Aldolase A 1683