STRUCTURAL EVIDENCE FOR A LIVER-SPECIFIC GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

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Received September 2,1975

SUMMARY: The amino acid sequences near the amino termini of glyceraldehyde-3-phosphate dehydrogenase from bovine and porcine liver have been determined. Using classical peptide isolation techniques as well as automated Edman degradation, the NH_2-terminal 30 residues of the bovine liver enzyme were determined to be Val-Lys-Val-Gly-Val-Asn-Gly-Phe-Gly-Arg-Ile-Gly-Arg-Leu-Val-Thr-Arg-Ala-Ala-Phe-Asn-Ser-Gly-Lys-Val-Asp-Ile-Val-Phe-Ile. Twenty-two residues from the NH_2-terminus of the porcine liver enzyme, determined using the automated Edman degradation, were identical to the corresponding sequence from bovine liver enzyme. Both liver enzymes have Asn at position 6. The corresponding residue 6 in the muscle and yeast glyceraldehyde-3-phosphate dehydrogenases is Asp. This evidence suggests that the Asn-6 residue is specific for the liver tissues. The exchange of Asn for Asp may significantly alter the allosteric properties of muscle and liver enzymes especially the activity of the liver enzymes in gluconeogenesis.

GAPDH[1] (EC 1.2.1.12) consists of 4 chemically identical subunits (1,2). The primary structures of the enzymes from yeast (3), pig (4) and lobster muscle (5) are known and, in spite of their isolation from distant species, show a high degree of homology (3). However, several pieces of evidence suggest that the GAPDH in the liver, a tissue involved in gluconeogenesis, may differ in its regulatory properties from the enzymes in muscle, a non-gluconeogenetic tissue. First, the muscle enzyme exhibits a negative co-operativity in coenzyme binding where in the affinity of NAD^+ binding progressively decreases with the addition of each of the four coenzyme molecules (6). The yeast (7) and liver enzymes, however, have a positive co-operativity in co-

[1]Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PTH, 3-phenyl-2-thiohydantoin; AE-, aminoethyl-; and CM-, carboxymethyl-.

enzyme binding. This discrepancy, in the case of liver enzyme, may reflect its function in gluconeogenesis. Second, tissue specific types of GAPDH and a neighboring enzyme in the metabolic pathway, 3-phosphoglycerate kinase (EC 2.7.2.3), have been reported (8-10). Third, GAPDH and 3-phosphoglycerate kinase catalyze ATP production in muscle. Reconstitution of the same enzymes from the liver favors gluconeogenesis under their optimal catalytic conditions (K. D. Kulbe, unpublished results). Finally, interesting differences between GAPDH from muscle and liver tissues were seen in the number of sulfhydryl groups, NAD^+ binding, allosteric properties and regulatory effects exerted by inorganic phosphate (8).

The amino acid sequence (4,5) and X-ray crystallography (11) have been revealed for muscle GAPDH. In order to understand the structure-function relationships, particularly the differences in allosteric responses, we have initiated sequence determinations of GAPDH from bovine, human and pig liver (12). This report presents part of our initial studies of the elucidation of the NH_2-terminal amino acid sequence of bovine and porcine liver GAPDH. The reliability and expedience of the new technique for multisample identification of phenylthiohydantoin-amino acids (13,14) also was tested in connection with automated sequence work.

METHODS

Enzyme purification: Bovine liver GAPDH was prepared as described (8) with improvements which consisted of replacement of DEAE-cellulose by DEAE-Sephadex A-50 for ion-exchange chromatography and a final gel filtration on Bio Gel P-150 (3 x 150 cm column). The pig liver enzyme was purified by affinity chromatography (H. Foellmer, E. Rieke, K. D. Kulbe, and W. Lamprecht, manuscript in preparation). Both proteins were homogeneous by sodium dodecyl sulfate electrophoresis (15) and sequence analysis to be reported here.

Tryptic digestion: Carboxymethylated bovine liver GAPDH (16) was digested, at a ratio of 50:1, by L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin in 0.1 M NH_4HCO_3 buffer, pH 8.2. After 6 hours the pH of the digest was adjusted to 3.5 with acetic acid. The solution was applied on a column of Sephadex G-25 (2.5 x 200 cm) and eluted with 0.05 M NH_4 OH. From pools D-F (see Fig. 2) small arginine-containing peptides were isolated by gel filtration on Sephadex G-10 gel (1.5 x 150 cm) and by high voltage electrophoreses at pH 6.5 and 2.0. For the isolation of lysine-overlapping tryptic peptides, the protein was reacted with maleic anhydride (17),

digested as described above and the peptides separated on a column of Bio Gel P-2 (2.5 x 200 cm) in 0.1 M NH_4HCO_3, pH 8.2. The NH_2-terminal peptide was purified by ion-exchange chromatography of demaleylated pool C on phosphocellulose (1.5 x 25 cm) (18). Using the same techniques, the Sakaguchi-positive neutral peptide MT-C-4-2 was isolated from yeast GAPDH (Boehringer). Molecular weight, amino acid composition ($Asp_{1.1}$ $Gly_{1.95}$ $Ala_{0.93}$ $Ile_{0.94}$ $Phe_{0.89}$ $Arg_{1.0}$) and the manually determined partial sequence Val-Ala-Ile-Asp-Gly demonstrate its identity to residues 3-10 in the yeast GAPDH structure (3). Electrophoretic mobilities at pH 6.5 were determined in relation to aspartic acid (R_{Asp}). Tryptic digestion of demaleylated MT-C-3-5 yielded two basic peptides with R_{Asp} values of 0.26 (MT-C-3-5-1) and 0.73 (MT-C-3-5-2).

Automated Edman degradation: Automated sequencial degradation was performed with aminoethylated (19) bovine and porcine liver GAPDH using a Beckman 890 C sequencer with program No. 040374 (fast protein-dime thyl allyl amine). A 90 sec extraction with ethyl acetate was added to this program at the end of the coupling and drying steps. PTH-amino acids were identified by micropolyamide thin-layer chromatography using either the one-dimensional double-development technique for simultaneous multi-sample identification or the two-dimensional method for single samples (13, 14). Most of the PTH-amino acids were quantitated by gas-liquid chromatography (20, 21). Arginine-PTH was identified from the aqueous phase by thin-layer chromatography.

Manual sequence analysis: Small arginine-containing peptides were analyzed by the dansyl-Edman technique (22) and amino acids from the whole protein were also identified at sub-nanomolar level on 5 x 5 cm micropolyamide sheets (13, 14). Peptide MT-C-3-5 was sequenced by direct Edman degradation in combination with the micropolyamide technique.

Amino acid analysis: Acid hydrolysis was carried out in 6 N HCl at 110^0 for 24 hours prior to analysis on a Beckman Unichrom amino-acid analyzer using the single column system (23).

RESULTS AND DISCUSSION

The 30-residue NH_2-terminal sequence of GAPDH from bovine liver was established as shown in Fig. 1. Each of the first 17 residues was identified by using an automatic sequencer on aminoethylated GAPDH as well as by determining the sequences of the peptides. The first 5 residues were also confirmed by manual Edman degradation of a sample of CM-GAPDH in the presence of sodium dodecyl sulfate (24). Residues 18-30 were identified in the automatic sequencer alone.

Several tryptic peptides of liver GAPDH were purified and sequenced. Peptides T7-A-1 and T7-A-2 were found to be Ile-Gly-Arg and Leu-Val-Thr-Arg. These sequences are apparently derived from residues 11-13 and residues 14-17 from the NH_2-terminal sequences shown in Fig. 1. Purification of the peptides was achieved on a column of Sephadex G-25 (Fig. 2). In this study only peptides from pools D-F were

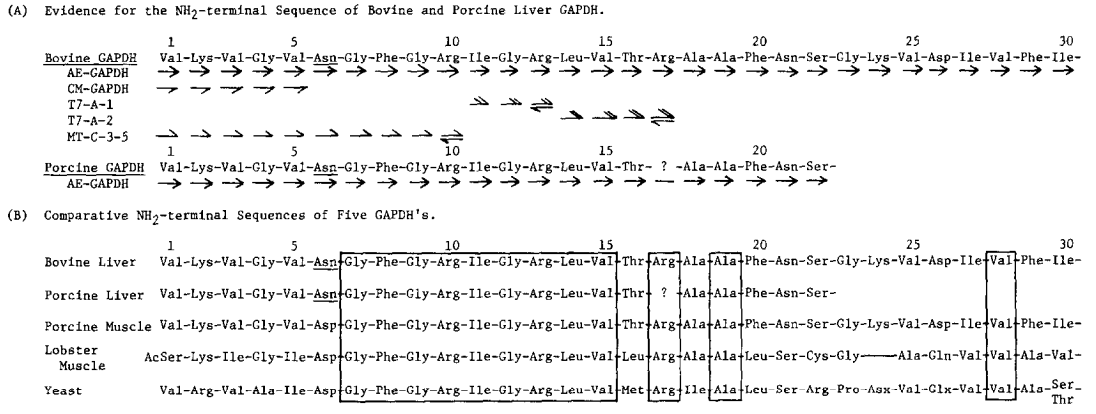

Fig. 1. The NH_2-terminal sequence of GAPDH from bovine and porcine liver. (A) Evidence for the new sequences. (B) Comparison of five known NH_2-terminal sequences of GAPDH's. In part (A) determinations were carried out by different techniques for degradation and residue identification: → automated Edman degradation with PTH-analysis by gas-liquid and polyamide TLC; —— no observation; → manual sodium dodecyl sulfate dansyl-Edman procedure plus PTH-detection by TLC; ⇒ manual dansyl-Edman degradation; → manual Edman procedure with PTH-identification by TLC; ← carboxypeptidase B treatment. The boxed residues in the comparative sequences (B) are the conserved residues.

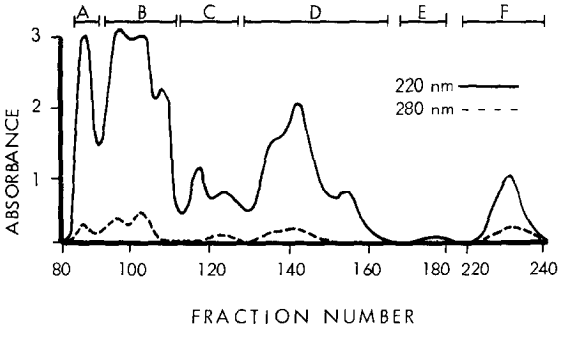

Fig. 2. Chromatographic pattern of a tryptic digest of carboxymethylated bovine-liver glyceraldehyde-3-phosphate dehydrogenase on a column of Sephadex G-25 (2.5 x 200 cm). Five ml fractions were collected. Arginine-positive small peptides were isolated from pools D-F.

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further examined. In addition to the two peptides described above, two additional peptides, T7-A-3 and T7-A-4 were purified and found to be Leu-Trp-Arg and Asp-Gly-Arg. The sequences are apparently identical, respectively, to residues 192-194 and residues 195-197 of the pig muscle protein (4). Figure 1 also contains 21 residues

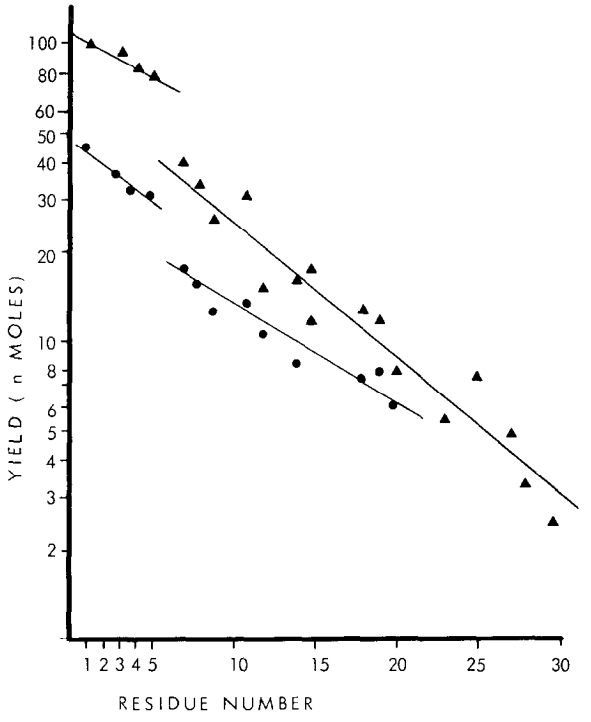

Fig. 3. The yields of PTH-amino acids derived from two separate automated Edman degradation of the aminoethylated bovine liver glyceraldehyde-3-phosphate dehydrogenase. The data were calculated from the peak heights of the gas-liquid chromatography, uncorrected for recovery losses.

from the NH_2 -terminus of porcine liver GAPDH which was determined only automatically.

Using the previously published TLC technique (13, 14) for PTH-identification in combination with quantitative results for hydrophobic residues from gas-liquid chromatography, we positively identified 30 residues of the bovine and 21 residues of the porcine enzyme. Residue 17 in porcine liver enzyme was not identified and remains unknown. Identification of Lys, Asn, Thr and Arg was either greatly facilitated or made possible by micropolyamide TLC. Figure 3 shows the semilogarithmic plots of the yield for most steps of two series of determinations in the automated Edman degradation of bovine liver GAPDH. In both experiments, the yields of residues after No. 7 dropped about 50%, as judged from the declining line of the first 6 residues. This was probably due

to an Asn-Gly rearrangement to a ß-peptide linkage, which cyclized and blocked further degradation (25).

The differences between the NH_2 -terminal sequences of GAPDH are summarized in Fig. 1. It is clear that the only difference between two porcine GAPDH's is the replacement of residue 6, Asn in liver enzyme and Asp in muscle enzyme. The relative mobility of deblocked TM-C-4-2 from yeast GAPDH (R_{Asp}= 0.02) and its partial sequence Val-Ala-Ile-Asp-Gly clearly indicate that this position is occupied by Asp. The corresponding peptide from bovine liver enzyme, TM-C-3-5-1, has an R_{Asp} value of 0.26 which confirms the presence of Asn. For any of the known GAPDHs from muscle tissues and bacterial sources there is no evidence at all for the presence of an Asn in position 6 (personal communication Dr. J. Walker, Cambridge/England). Therefore, the fact that residue 6 in bovine and porcine liver is Asn and that this residue in yeast, porcine and lobster muscle is Asp suggests that the Asn ist specific for liver GAPDH. A comparison of NH_2- terminal sequences for all GAPDHs (Fig. 1) indicates that a total of 13 residues (43%) are invariant. Eleven of the 17 differences can be ascribed to point mutations and 6 to two base changes. Bovine liver GAPDH differs from the lobster and yeast enzymes, in the NH_2 -terminal 30 residues, at 13 (57% homology) and 14 positions (53% homology) respectively. A comparison of this same segment of the known sequences in muscle enzyme from pig and lobster shows a homology of 53%. The complete sequences of the two protein chains show 72% of identical residues in the corresponding positions.

A detailed X-ray study of lobster muscle GAPDH shows that residues 1-149 are associated with the binding of coenzyme (11). The folding in this domain is similar to the corresponding functional domains in other dehydrogenases (26, 27). The NAD^+ binding site consists of a six-stranded parallel β-sheet with the strand order CBADEF. From this structural information, the Asp/Asn interchange between muscle and liver GAPDH appears to be remarkable since this residue is situated near the carboxyl end of the strand βA, which forms together with strands βB and βD the central region of the

sheet. This region is part of the coenzyme binding domain which functions as an adenosine subsite. The NH_2-terminal 30-residue structure is part of strands βA and βB and helix αB, which provides 5 (residues 6,10,13,18 and 22) of the 29 side chains involved in the active center region (11). In comparison to muscle GAPDH, Asn-6 as a member of the βA strand in liver GAPDH could influence the binding stability for the adenosine phosphate part of the coenzyme molecule and may explain the weak interaction of NAD^+ with the bovine liver enzyme in the crystal (8). In glutamate dehydrogenase from bovine (28) and chicken liver (29) the corresponding position 250 is also occupied by the neutral Gln residue, whereas the enzyme from Neurospora crassa has a serine side chain in this position. The allosteric properties and coenzyme specificity are, however, different for both types of enzymes.

The above results indicate that the observed Asp/Asn exchange at position 6 is tissue specific rather than a species difference. The structure of GAPDH from liver of other species will be needed to further confirm this point. An interesting question remaining unanswered is whether this Asp/Asn exchange is also specific for its function in gluconeogenesis. In an attempt to further clarify this point, we are currently studying the GAPDH from bovine and porcine kidney.

ACKNOWLEDGEMENT

This work was supported in part by a travel grant (Ku 336/2) from Deutsche Forschungsgemeinschaft and in part by NIH Research Grant AM-06487.

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