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AMINO ACID SEQUENCE OF AN INVERTEBRATE FBP ALDOLASE (FROM DROSOPHILA MELANOGASTER)

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SUMMARY: The complete amino acid sequence of FBP aldolase from Drosophila melanogaster has been determined. The enzyme contains four identical subunits of 360 amino acid residues. The primary structure of the monomer was established using automated Edman degradation on fragments prepared by CNBr-cleavage, by partial acid cleavage at the unique Asp-Pro bond and by oxidative cleavage at the three tryptophan residues. Manual Edman-Chang degradation was used on smaller peptides obtained by digestion with Staphylococcus aureus V8 protease, trypsin or chymotrypsin.

The primary structure of Drosophila aldolase exhibits very extensive homology with the sequence of rabbit muscle aldolase (71% identity), thus explaining the early observation that Drosophila and mammalian aldolases form active interspecies hybrid quaternary structures (Brenner-Holzach, O. and Leuthardt, F., Eur. J. Biochem. (1972) 31, 423-426).

FBP aldolase (EC 4.1.2.13) from Drosophila melanogaster is a class I aldolase with features close to those of vertebrate aldolases (1,2). It has the same molecular weight and the same quaternary structure of four subunits (3). The tertiary structure of the subunits are also thought to be very similar since enzymatically active quaternary structure hybrids can be formed between Drosophila aldolase and certain mammalian aldolases (4). This close structural relatedness is also indicated by partial sequence studies which showed extensive homology to the published amino acid sequences of rabbit muscle FBP aldolase (5,6).

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Abbreviations: FBP, fructose 1,6-bisphosphate; CNBr, cyanogen bromide; TFA, trifluoro acetic acid; DABTH, 4-N,N-dimethylaminobenzene 4'-thiohydantoin; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; DALD, Drosophila aldolase; MALD, rabbit muscle aldolase.

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We have recently reported that FBP aldolase from Drosophila yields crystals suitable for high-resolution X-ray analysis (7). To facilitate the completion of the crystallographic work now in progress it is desirable to know the entire chemical structure of the enzyme. In the present paper, we report the complete amino acid sequence of Drosophila FBP aldolase.

MATERIAL AND METHODS

Staphylococcus aureus V8 protease was purchased from Miles Laboratories. Trypsin (TPCK) was a product from Worthington or Boehringer, Chymotrypsin from Sigma. o-Iodobenzoic acid was obtained from Pierce. For HPLC separations, columns were used from Brownlee (Lichrosorb, RP-18 or RP-300) and Du Pont (Zorbax ODS and CN).

Isolation of Drosophila aldolase: Drosophila aldolase was isolated and purified employing a method previously described (4).

Sequence analyses were performed on peptides prepared by various chemical cleavage procedures and consecutive subdigestions with endoproteases (see Fig. 2): The carboxymethylated protein was cleaved with CNBr in 70% HCOOH for 24 h at 30° C (8) and after citraconylation the fragments were purified on Sephadex G-75 (9). Partial acid cleavage at the Asp-Pro bond of the enzyme was achieved in 70% HCOOH for 48 h at 37° C and the fragments were separated on Sephadex G-200 with 50% HCOOH. Cleavage at tryptophan residues was attained by oxidation of the native or carboxymethylated protein with o-iodobenzoic acid following the method of Mahoney et al. (10). The fragments were purified on Biogel P-30 in 10% HCOOH.
Subdigestion of larger peptides was carried out after citraconylation with Staphylococcus aureus V8 protease (in 0.5% NH_4HCO_3 buffer, pH 8.0, or in 0.2 M ethylmorpholine buffer, pH 8.0, at an enzyme-to-substrate ratio of 1 : 50, 15 - 18 h, 37° C), with trypsin (TPCK, in 0.5% NH_4HCO_3, pH 8.0, at an enzyme-to-substrate ratio of 1 : 50, 1.5 h, at 37° C) and with chymotrypsin (same conditions as for trypsin). Specific cleavage at arginine residues with trypsin was accomplished after citraconylation of the ε-amino groups of lysyl residues (9).
The peptides resulting from subdigestions were separated by HPLC on a reversed-phase column (Lichrosorb RP-18 or RP-300), with a TFA/acetonitril buffer system (0.1% TFA and 0.1% TFA / 60% acetonitril).
Amino acid analyses were performed on a Durrum amino acid analyzer (Model D-500).
Depending on the size of the peptides sequence analyses were carried out by either automated or manual Edman degradation techniques, the latter with modifications published by Chang (11). The DABTH derivatives were identified by HPLC on a Zorbax ODS or CN column (Du Pont) or by TLC on polyamid sheets (Schleicher & Schuell) (11).
Automated sequence analysis was performed on a Beckman Spinco sequencer model 890 C using the quadrol (0.25 M) program together with Polybren (Sigma) as an additive (12). Phenylthiohydantoin amino acid derivatives were identified by isocratic normal phase HPLC which was a slight modification of the system described in (13). The main differences are the use of Partisil PAC (Whatman) for the polar group and a third column for the identification of His and Arg.

The latter was Partisil PAC eluted with a solvent mixture of methylene chloride, methanol, and dioxane which contained ammonium acetate.

RESULTS AND DISCUSSION

Figure 1 presents the complete primary structure of the monomer of FBP aldolase from Drosophila melanogaster (Sevelen, $^{+}/^{+}$) and compares it with the known sequence of the rabbit muscle FBP aldolase monomer (5,6). The strategy employed for sequencing the Drosophila enzyme is outlined in figure 2. Most studies were conducted on the S-carboxymethylated derivative. Large fragments were generated by CNBr-cleavage at the three methionine residues, by partial acid cleavage at the sole Asp-Pro bond and by oxidative cleavage at the three tryptophan residues using o-iodobenzoic acid. The resulting peptides were separated by gel filtration. Shorter fragments were prepared by subdigestion with Staphylococcus aureus V8 protease, trypsin or chymotrypsin and were separated by HPLC chromatography. Some overlapping peptides were provided by tryptic and chymotryptic cleavage of citraconylated CNBr-peptides.

Amino acid sequence determinations of the peptides were carried out by automated and manual Edman degradation. The sequence of the blocked N-terminal chymotryptic peptide was established by g.l.c.-mass spectrometry following per-

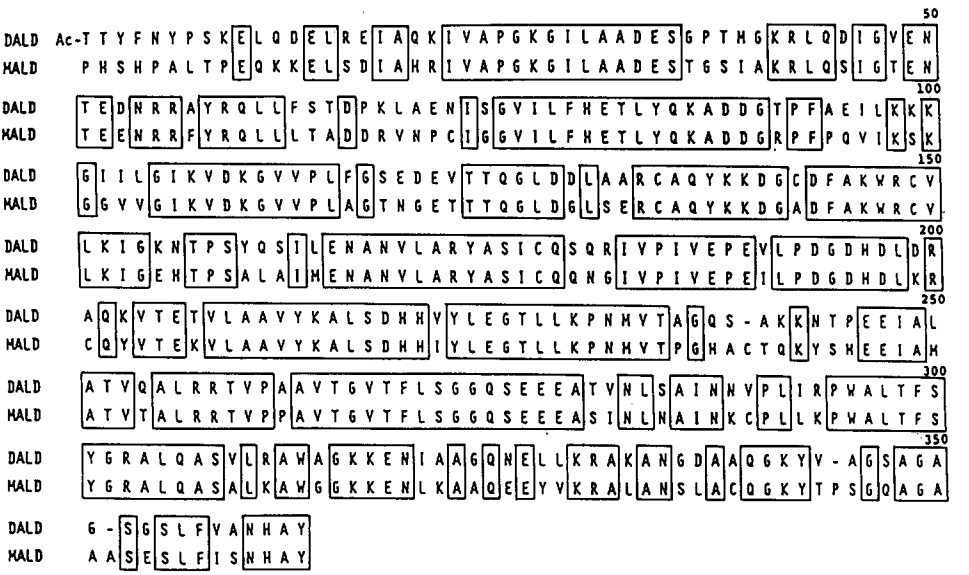

Fig. 1. Amino acid sequences of FBP aldolase from Drosophila melanogaster (DALD) and from rabbit muscle (MALD). The numbering refers to that of the rabbit FBP aldolase sequence (6). The framed portions indicate identical segments in the two enzymes. LYS-229 is the substrate-binding lysyl residue (1,14).

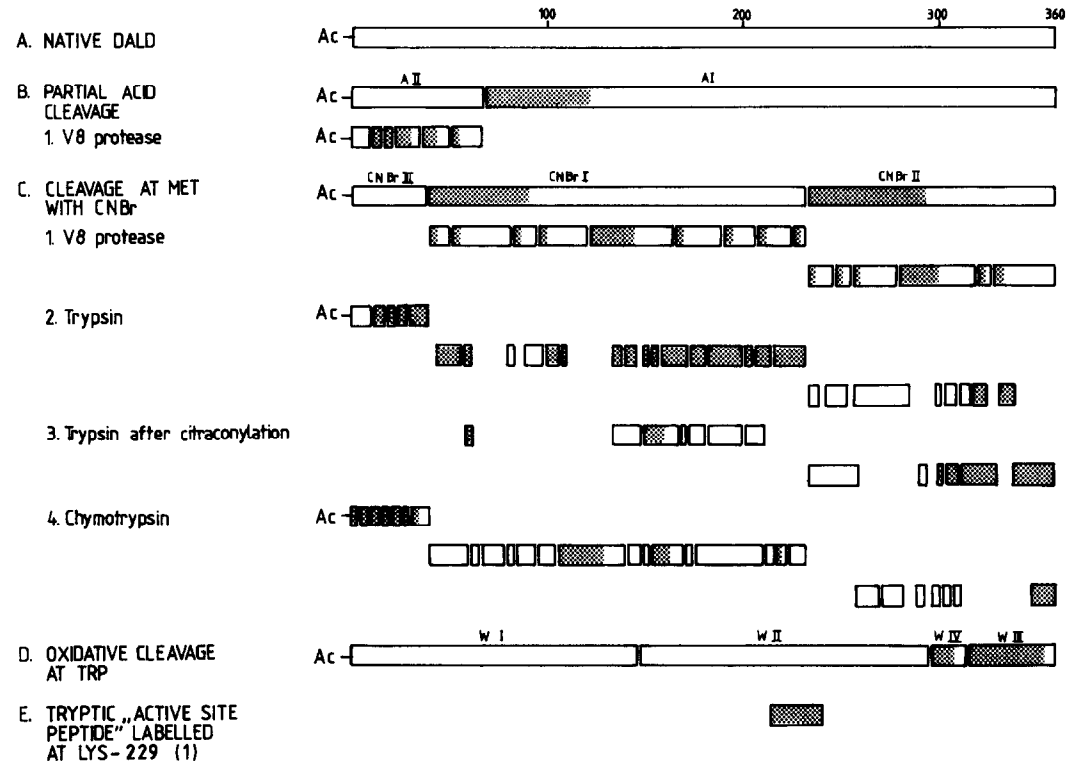

Fig. 2. Strategy of the amino acid sequence determination of Drosophila aldolase. The top bar represents the entire monomer polypeptide chain (A). The broken bars show the fragments generated by partial acid cleavage at the single Asp-Pro bond (B), by CNBr cleavage at the Met residues (C), and by oxidative cleavage at the Trp residues (D). The smaller peptides were prepared by subdigestion of the larger fragments with endoproteases (Staphylococcus aureus V8 protease, trypsin or chymotrypsin). The shadowed areas of the bars represent the sequenced portions either by automated sequence determination or by manual Edman-Chang degradation (11).
AI/AII are peptides from partial acid cleavage, CNBrI-III peptides from cleavage with cyanogen bromide, and WI-WIV peptides from oxidative cleavage at tryptophan residues.

methylation (Rose et al., in preparation). For each cleavage point, there were overlaps by at least two residues allowing an unambiguous alignment of all peptides. A complete account of the sequencing data will be published subsequently.

Based on the sequence, the monomer of the Drosophila FBP aldolase contains a total of 360 amino acid residues and has a chain weight of 38914. The amino acid composition is nearly identical with previously reported amino acid analyses data of the enzyme (Table 1). The only significant difference relates to the number of tryptophan residues where earlier measurements indicated four residues instead of the three now documented (3). There is also a complete

Table 1

Amino acid composition of Drosophila aldolase, as derived from analysis of the acid hydrolysate (3), from the sum total of the amino acids contained in the Staphylococcus aureux V8 protease peptides recoverd and from the total sequence.

Amino acid	Amino acid recovery from acid hydrolysate	Sum of amino acids of V8 peptides recovered	Sum of amino acids from sequence
D	36	35	19
N	-	-	16
T	21	21	21
S	20	19	19
E	42	40	23
Q	-	-	17
P	15	15	15
G	29	29	29
A	43	44	44
CM-C	4	4	4
V	26	26	26
M	2	2	2
I	19	18/19	19
L	35	35	35
Y	11	12	12
F	10	9	9
H	5	5	5
K	26	26	26
R	16	16	16
W	4/3	3	3
Number of residues	364/363	359/360	360

agreement between the amino acid composition of the chain and the sum of amino acids in the 20 peptides obtained after digestion with Staphylococcus aureus V8 protease. The correspondence and the absence of supernumerary variant peptides implies that Drosophila FBP aldolase is a homotetrameric protein composed of a single type of subunits.

The most striking result of this study is the similarity between the primary structure of Drosophila and rabbit muscle FBP aldolases. The two sequences are identical in 71% of all residues and many of the nonidentical residues are chemically homologous. The structural similarity (95% identity) is highest in the active site region (positions 208-234) (1,5,14,15). This extent of sequence correspondence is remarkable in view of the large evolutional distance of insects from mammals. Since very close similarities have also been found between

mammalian and fish FBP aldolases (1,16), it probably reflects an intrinsically low rate of molecular evolution of this enzyme. The high sequence homology of the insect and mammalian FBP aldolases ensures a similar mode of folding of their polypeptide chains and thus affords a satisfactory explanation for their above-mentioned capacity to form interspecies hybrid quaternary structures (4).

The largest difference between the two sequences occurs in the N-terminal region. There is no obvious homology in the first nine residues and, in contrast to rabbit muscle aldolase, the N-terminus of Drosophila aldolase is blocked by an acetyl group. Compared to the mammalian enzyme, the polypeptide chain of the insect aldolase is shorter by three residues. Using the numbering employed for rabbit muscle aldolase by Tolan et al. (6), Drosophila aldolase lacks a residue in position 239, 344 and 353. There are also differences in the total number of acidic amino acid residues. While both polypeptide chains carry nearly the same number of strongly basic residues (K+R = 42 for DALD, K + R = 41 for MALD), Drosophila aldolase contains more acidic residues (D + E = 42) than rabbit muscle aldolase (D + E = 38), in agreement with the lower isoelectric point of the insect enzyme.

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