AMINO ACID SEQUENCE HOMOLOGY BETWEEN MUSCLE AND LIVER ALDOLASES

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1. Introduction

A detailed knowledge of the primary structure of the same protein isolated from a variety of species and from different tissues in the same specimen is desirable for an understanding of the genetic and functional relationships which have developed both in phylogenesis and in ontogenesis. Useful information can often be obtained from a comparison of selected regions of the protein molecule. Thus, it has been shown that a remarkably high degree of homology exists in the amino acid sequence around the substrate-binding lysine residue of fructose diphosphate aldolase [E.C. 4.1.2.13] isolated from the muscle of distantly related sources such as mammals [1–3], amphibia [4] and fish [3, 5]. The degree of homology is entirely comparable with that found for another muscle glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase [6, 7].

It has been suggested that such homology will be a common feature of the glycolytic enzymes [8]. We have therefore sought to establish whether comparable homology exists for the glycolytic enzymes isolated from tissues other than muscle. We show here that there exists an identical amino acid sequence (36 residues) around the active site lysine residue of aldolase from the liver of rabbit and of ox. These results are in complete agreement with the corrected amino acid sequence for part of this region which appeared [9] during the preparation of this manuscript.

Abbreviations:
Cmc: *S*-carboxymethylcysteine
Hsr : Homoserine

2. Materials and methods

Crystalline aldolase from fresh rabbit and beef liver was prepared by the method of Penhoet et al. [10] and *S*-carboxylmethylated with [2-^{14}C]-iodoacetic acid in 8 M urea as described previously [8]. Cyanogen bromide cleavage was carried out in 70% formic acid for 24 hr at room temperature and the resulting peptides were reacted with citraconic anhydride [11, 12] to promote solubility of the larger fragments. Preliminary separation of the citraconylated peptides was achieved by gel filtration on a column of Sephadex G-75 Superfine (140 × 2.5 cm) in 0.5% NH_4HCO_3, pH 8. The various peaks from the Sephadex column, after having been freeze-dried, had their citraconyl groups removed by incubation overnight in 5% formic acid at 20°.

Peptides (20–50 nmoles) were digested completely with aminopeptidase M (PL Biochemicals Inc., Milwaukee, Wisconsin, USA) by dissolving the peptide in 0.1 ml of 50 mM-*N*-ethylmorpholine-acetic acid buffer, pH 8, adding 10 μg of enzyme and leaving 16 hr at 20°. Other enzymic digestions, paper electrophoresis, amino acid analysis and dansyl-Edman degradation of peptides were carried out as previously reported [3]. The electrophoretic mobility (m) of a peptide at pH 6.5 was used to help establish amide assignments [13].

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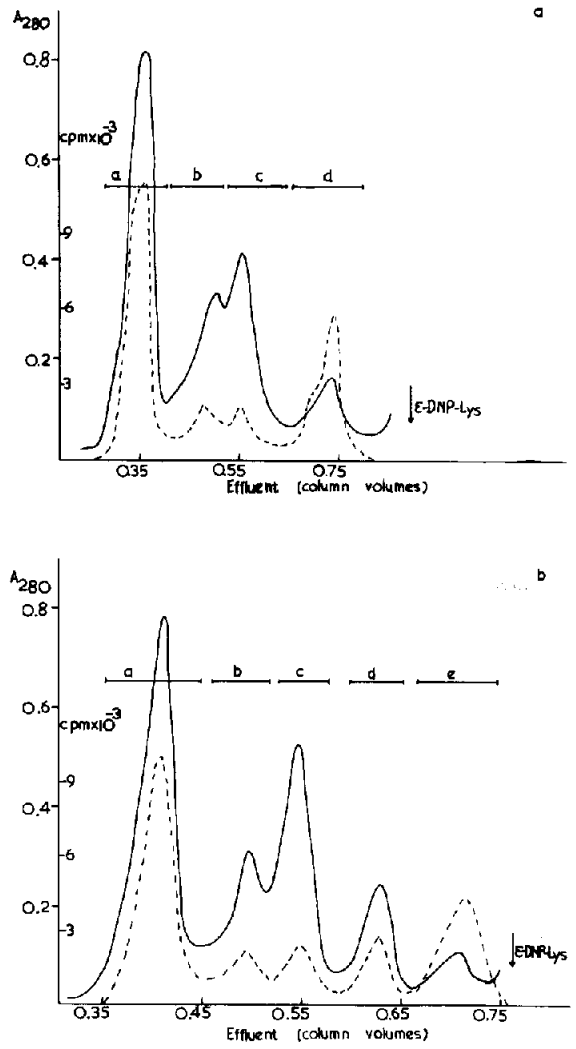

Fig. 1. Elution profile of the cyanogen bromide peptides of (^{14}C) *S*-carboxymethylated aldolase from rabbit (a) and ox (b) liver on Sephadex G-75. - - - - - c.p.m.; ——— A_{280}.

3. Results

It has previously been shown that in the muscle aldolases the degree of homology around the substrate-binding lysine residue is such as to permit unambiguous identification of the active site tryptic peptide by amino acid analysis. Moreover, the presence of two histidine residues in the peptide can be exploited as a "handle" in the purification procedures, thus avoiding the necessity of labeling the active site with substrate [3]. An analogous approach was therefore used in the present work in the hope that the same situation would obtain for the liver aldolases.

The elution profiles from Sephadex G-75 of the cyanogen bromide digest of rabbit and ox liver *S*-carboxymethylated aldolases are shown in fig. 1. Tryptic digestion of peak RXa from the rabbit liver enzyme followed by electrophoresis at pH 6.5 and pH 3.5 allowed the purification of a peptide, RXaT7, having the amino acid composition: Lys_1, His_2, Asp_3, Thr_1, Glu_1, Pro_1, Gly_1, Ala_1, Val_1, Leu_4, Tyr_1, Hsr_1. Alanine was shown to be *N*-terminal by the dansyl procedure. After further digestion of peptide RXaT7 with chymotrypsin the following peptides were separated by paper electrophoresis at pH 6.5 and pH 3.5; peptide C_1: Thr_1, Glu_1, Gly_1, Leu_3 (m = −0.40) peptide C_3: His_2, Asp_2, Ala_1, Val_1, Leu_1, Tyr_1 (m = +0.18) and peptide C_4: Lys_1, Asp_1, Pro_1, Hsr_1 (m = +0.44). The alignment of these peptides was established on the assumption that peptide C_4 must derive from the *C*-terminus of peptide RXaT7 since it contains homoserine and peptide C_3 from the *N*-terminus since it contains alanine as *N*-terminal residue. Dansyl-Edman degradation then enabled a unique sequence to be established for peptide RXaT7 as shown in fig. 2.

In order to establish unequivocally the amide assignments in peptide C_3, the following approach was used: 20 nmoles of peptide were digested with aminopeptidase M for 16 hr at 20°. Half the digest was applied to the automatic amino acid analyser while the remainder was submitted to paper electrophoresis at pH 6.5 and pH 2 and then stained with ninhydrin-collidine. With both methods the presence of asparagine and aspartic acid was established. Digestion of peptide C_3 with aminopeptidase M after three rounds of Edman degradation released only aspartic acid, showing therefore that the sequence must be Ala–Leu–Asn–Asp–.

Although the elution profiles of the CNBr fragments of the rabbit and ox liver aldolases from Sephadex G-75 are essentially the same (fig. 1), the appearance of an extra peak, OXd, is a noteworthy difference for the ox enzyme. More interesting was the finding that from this peak the ox active site peptide, OXdT7, could be isolated by a technique identical to that used for the rabbit peptide RXaT7 above.

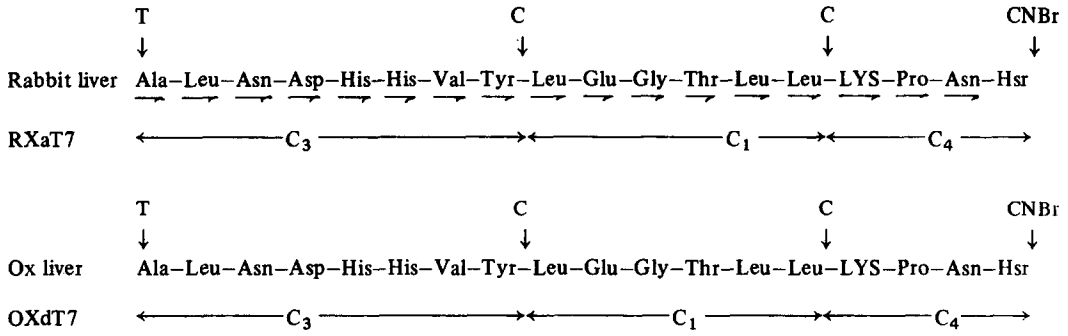

Fig. 2. The amino acid sequence of peptides RXaT7 and OXdT7 from rabbit and ox liver aldolases. CNBr ↓, T ↓, and C ↓ denote bonds cleaved by cyanogen bromide, trypsin and chymotrypsin, respectively; → denotes a residue established by the dansyl-Edman degradation.

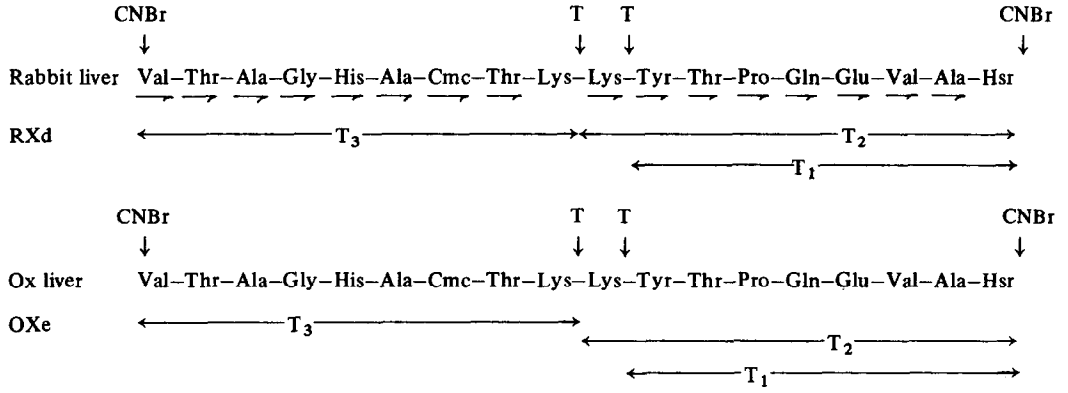

Fig. 3. The amino acid sequence of peptides RXd and OXe from rabbit and ox liver aldolases.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Rabbit muscle	–Ala–	Leu–	Ser–	Asp–	His–	His–	Ile–	Tyr–	Leu–	Glu–	Gly–	Thr–	Leu–	Leu–	LYS–	Pro–	Asn–	Met–	Val–	Thr–
Rabbit liver	–Ala–	Leu–	Asn–	Asp–	His–	His–	Val–	Tyr–	Leu–	Glu–	Gly–	Thr–	Leu–	Leu–	LYS–	Pro–	Asn–	Met–	Val–	Thr–
Sturgeon muscle	–Ala–	Leu–	Ser–	Asp–	His–	His–	Val–	Tyr–	Leu–	Glu–	Gly–	Thr–	Leu–	Leu–	LYS–	Pro–	Asn–	Met–	Val–	Thr–

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Rabbit muscle	Pro–	Gly–	His–	Ala–	Cys–	Thr–	Gln–	Lys–	Tyr–	Ser–	His–	Glu–	Glu–	Ile–	Ala–	Met–
Rabbit liver	Ala–	Gly–	His–	Ala–	Cys–	Thr–	Lys–	Lys–	Tyr–	Thr–	Pro–	Gln–	Glu–	Val–	Ala–	Met–
Sturgeon muscle	Ala–	Gly–	Gln–	Ala–	Cys–	Thr–	Lys–	Lys–	Tyr–	Thr–	Ser–	Gln–	Glu–	Ile–	Ala–	Met–

Fig. 4. The amino acid sequence around the substrate-binding lysine residue of aldolase from rabbit muscle, rabbit liver and sturgeon (*Acipenser fulvescens*) muscle. The substrate-binding residue is at position 15.

Chymotryptic digestion of peptide OXdT7 gave fragments C_1, C_3 and C_4 that were shown to be identical with those obtained from the rabbit peptide RXaT7, enabling the amino acid sequence of peptide OXdT7 to be formulated as shown in fig. 2.

It had already been shown [14] that the smallest CNBr fragment isolated from rabbit muscle aldolase is a peptide of 18 residues adjacent to the substrate-binding lysine residue in the active site and that the same situation holds for sturgeon muscle aldolase [3]. When peaks RXd and OXe from rabbit and ox liver aldolases respectively (fig. 1) were purified by paper electrophoresis at pH 6.5 and pH 3.5, a peptide with identical amino acid composition was isolated from both: Lys_2, His_1, Cmc_1, Thr_3, Glu_2, Pro_1, Gly_1, Ala_3, Val_2, Tyr_1, Hsr_1. Valine was found at the *N*-terminus by the dansyl procedure. After tryptic digestion of either peptide, the following fragments were separated by electrophoresis at pH 6.5 and pH 3.5; T_1: Thr_1, Glu_2, Ala_1, Val_1, Pro_1, Tyr_1, Hsr_1 (m = −0.30); T_2: Lys_1, Thr_1, Glu_2, Ala_1, Val_1, Pro_1, Tyr_1, Hsr_1 (m = 0.00); T_3: Lys_1, His_1, Cmc_1, Thr_2, Gly_1, Ala_2, Val_1 (m = + 0.25) and T_4: free Lys. The sequences of these peptides were established by the dansyl-Edman degradation and could be fitted together uniquely as summarised in fig. 3. Complete digestion of peptide T_1 with aminopeptidase M after three rounds of Edman degradation released both glutamic acid and glutamine, as judged by paper electrophoresis. After four rounds of degradation only glutamic acid was released by aminopeptidase digestion, thereby establishing the sequence as Tyr–Thr–Pro–Gln–Glu–.

The amino acid sequence of the *N*-terminal region of peptides RXd and OXe is identical with the *C*-terminal sequence of a tryptic peptide derived from the active site of rabbit liver aldolase previously published [15]. This is sufficient to prove that peptide RXd lies on the *C*-terminal side of peptide RXaT7 in the rabbit liver enzyme and, since the homology is so strong, to establish beyond reasonable doubt that peptide OXe lies on the *C*-terminal side of peptide OXdT7 in the ox liver enzyme (fig. 4).

4. Discussion

The amino acid sequences around the substrate-binding lysine residue of aldolase from rabbit muscle [3], sturgeon muscle [3] and ox and rabbit liver are shown in fig. 4. Although there are minor differences in amide distribution between the rabbit muscle enzyme sequence given in fig. 4 and other sequences recently published elsewhere [2, 16], there is complete agreement for the ox and rabbit liver enzyme sequence (residues 1–27) between this study and the amended version which has also recently been published [9]. The extension of the liver enzyme sequence from position 28 to position 36 is new.

The ox and rabbit liver enzymes have identical amino acid sequences for at least 36 residues in this region of the molecule. It seems clear then that the impressive sequence homology previously demonstrated for the aldolases of muscle [8] will also be found for the aldolases of liver. A few differences between the ox and rabbit liver enzymes obviously do exist, as shown by the difference in cyanogen bromide cleavage (fig. 1) and by detailed study of other tryptic peptides (B.G. Forcina & R.N. Perham, unpublished work). However, it is interesting that the high degree of sequence homology in aldolases is maintained for the enzymes from liver.

It is also evident from fig. 4 that, in this region at least, the aldolases from rabbit muscle and rabbit liver are no more alike than those from rabbit muscle and sturgeon muscle. In fact, there are fewest changes between the enzymes from rabbit liver and sturgeon muscle. Speculation about the significance of this is probably premature since the liver and muscle enzymes come from functionally distinct tissues, one gluconeogenic and the other glycolytic: thus the selection pressures on the two enzymes are probably significantly different. This is reflected in the different specificities of the liver and muscle enzymes [17].

It is unfortunate that no explanation for the difference in specificity of the liver and muscle enzymes can be seen in the sequences of fig. 4. However, it is probably unreasonable to suppose that it should, since the active site of the enzyme will contain structural components outside the sequences established for the enzymes in the present study. Nonetheless, the degree of homology between the muscle and liver

aldolases is such that there is every reason to believe that the enzymes will have similar three-dimensional structures and to hope that a model established by X-ray crystallography for the muscle enzyme [18] will allow a reliable model for the liver enzyme to be built from a knowledge of its sequence alone.

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