PRIMARY STRUCTURE OF THE ESSENTIAL THIOL PEPTIDE FROM THE LACTATE DEHYDROGENASE C SUBUNIT

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Received November 1,1976

SUMMARY. Lactate dehydrogenase isozymes are inhibited when mercurial reagents are bound to cysteine-165 although no functional role is ascribed to this residue. Identical tryptic peptides containing this cysteine have been isolated from many LDH isozymes, including both A and B subunits. This report identifies an identical peptide from a third subunit type, C, of mouse. The rigorous conservation of this sequence implies an important functional role for this region of the molecule.

INTRODUCTION

The inhibition of lactate dehydrogenase (L-lactate:NAD oxidoreductase, E.C. 1.1.1.27) (LDH) by mercurial compounds and other sulfhydryl reagents has been the subject of numerous studies (1). Many LDH isozymes are inactivated upon binding 4 molecules of mercurial compound per tetramer (2). The reagent is bound to a cysteine residue, and an "essential thiol" tryptic peptide of highly conserved sequence containing this cysteine has been isolated from both LDH-A and LDH-B subunits of seven species (2,3,4,5).

In addition to the A and B polypeptides found in all tissues of vertebrates, a third type of subunit, LDH-C, is found in mature testes and spermatozoa of mammals. LDH-C is chemically distinct from the A and B subunits and is encoded by a third genetic locus. The C_4 homotetramer is known as LDH-X (6). LDH-X has been purified to homogeneity from mouse testes (7). In contrast to other LDH isozymes, mouse LDH-X activity is only 50% inhibited upon binding p-hydroxymercuribenzoate at a 4:1 molar ratio. The activity is only slightly reduced with additional reagent up to the point at which the enzyme precipitates (7). This suggests that the essential cysteine is lost, that its reactivity is changed, or that there is a structural alteration in active site around this residue. A tryptic peptide containing the "essential thiol" residue of mouse LDH-X is described here.

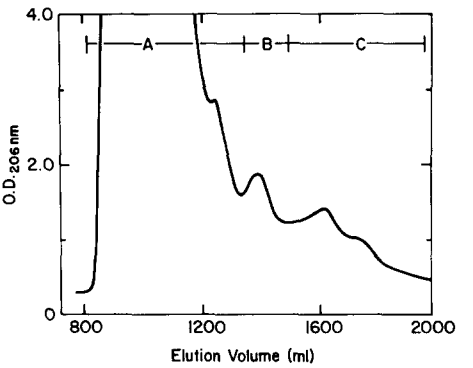

Figure 1. Chromatography of the limited tryptic digest of mouse LDH-X on a 5 x 125 cm Sephadex G-50 column. Elution is with 0.05 M NH_4OH at 3 ml/min. The effluent is continuously monitored at 206 nM with an LKB Uvicord III. Fractions comprising peaks A, B, and C are pooled and lyophilized.

MATERIALS AND METHODS

Mouse LDH-X (265 mg) was carboxymethylated (8) and then citraconylated in 8 M urea (9). The modified LDH-X was exhaustively dialyzed against 0.05 M NH_4HCO_3. Digestion with L-(tosylamido 2-phenyl)ethyl chloromethyl ketone treated trypsin (Worthington), 1% w/w, proceeded for 4 hr at 37°C and was stopped with a two-fold excess of soybean trypsin inhibitor (Worthington). Peptides were purified by gel filtration chromatography on Sephadex G-50 and preparative paper electrophoresis at pH 6.5 (10). Amino acid analyses were performed on a Durrum D-500 analyzer following hydrolysis in 6 N HCl (Pierce) in vacuo at 108°C for 30 hr. Amino acid sequences were determined by both the dansyl-Edman procedure (11) and by automated Edman degradation (12) using a Beckman Model 890C sequencer with a modified peptide program. The phenylthiohydantoin amino acids generated by the latter procedure were directly identified by thin-layer chromatography (13).

RESULTS

Chromatography of the limited tryptic digest on Sephadex G-50 gave the elution profile shown in Figure 1. Peak B contained three major peptides which were subjected to preparative paper electrophoresis at pH 6.5. An acidic peptide was eluted from the paper with water. This peptide represented about half the material in peak B and approximately 600 nMoles was recovered. Two-dimensional thin layer peptide maps demonstrated its purity.

This peptide was sequenced by both the dansyl-Edman procedure and by automated Edman degradation using the Beckman Model 890C sequencer. Both

Table 1

Comparison of essential thiol peptides from vertebrate LDH isozymes

Mouse LDH-C	(Arg)Val-Ile-Gly-Ser-Gly-Cys-Asn-Leu-Asp-Ser-Ala-Arg
Dogfish LDH-A*	Arg Ile-Ile-Gly-Ser-Gly-Cys-Asn-Leu-Asp-Ser-Ala-Arg
Rabbit LDH-A*	(Arg)Val-Ile-Gly-Ser-Gly-Cys-Asn-Leu-Asp-Ser-Ala-Arg
Chicken LDH-B*	(Arg)Val-Ile-Gly-Ser-Gly-Cys-Asn-Leu-Asp-Thr-Ala-Arg
Pig LDH-B**	(Arg)Val-Ile-Gly-Ser-Gly-Cys-Asn-Leu-Asp-Ser-Ala-Arg
Beef LDH-B*	(Arg)Val-Ile-Gly-Ser-Gly-Cys-Asn-Leu-Asp-Ser-Ala-Arg

*According to Taylor and Oxley (5).
**According to Holbrook et al. (3).

methods gave the same sequence (Table 1) with no ambiguity at any position. Amides were placed by direct identification of the phenylthiohydantoin amino acid. This sequence fits well the experimentally determined amino acid composition of the peptide. The electrophoretic mobility of the peptide relative to aspartic acid is compatible with the calculated molecular weight (14).

DISCUSSION

The "essential thiol" peptide from the mouse LDH-C subunit has a sequence comparable to the homologous region of other vertebrate LDH subunits as shown in Table 1 (8). The peptide isolated from mouse LDH-X is probably preceded by an arginine because the lysines were blocked by citraconylation before tryptic digestion. All vertebrate LDH isozymes examined, now including three distinct subunit types, contain nearly identical "essential thiol" peptides. Lobster tail LDH also yields a tryptic peptide of the same sequence except that it lacks cysteine-165. Threonine is substituted for this cysteine and serine for alanine-170 (5). The rigorous conservation of this rather long sequence strongly suggests an important functional role.

With the known sequence (8,15) and three-dimensional configuration (16,17) of dogfish LDH-A, the structural basis of inactivation by mercurials can be examined. The "essential thiol" has been identified as cysteine-165 in dogfish LDH-A (15,18). Crystallographic studies of this isozyme suggest that cysteine-165 is not essential for catalysis and does not participate directly in coenzyme binding (17). However, this residue is in the substrate binding pocket, and bound mercurials probably inhibit activity by steric hindrance.

LDH-X shows a pattern of mercurial inhibition different from other LDH isozymes as noted above (7). The present data indicate that this is not due to the absence of a residue homologous to cysteine-165 or to changes in the sequence around this residue. In addition, X-ray crystallographic studies at a resolution of 7.5 Å reveal no major differences in conformation between mouse LDH-C and dogfish LDH-A (19). The complete amino acid sequence together with the three-dimensional structure may reveal that sequence variations elsewhere in the molecule lead to changes in the configuration of the substrate binding pocket which account for the particular pattern of inhibition of this isozyme by mercurials.

ACKNOWLEDGEMENTS: Automated Edman degradations were performed with the assistance of Drs. James Beecher and George Tarr. Amino acid analyses were performed with the support of NIH grant No. HL-11119. This research was supported by NIH grant No. RO1 HD05863. We appreciate the helpful discussion of Dr. S.S. Taylor and editorial assistance by P. Bentley.

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