

Review Article

Structure, Regulation and Evolution of Vertebrate Lactate Dehydrogenase Genes

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ABSTRACT

Steven Shoei-Lung Li (1998) Structure, regulation and evolution of vertebrate lactate dehydrogenase genes. *Zoological Studies* 37(1): 1-6. In vertebrates, L-lactate dehydrogenase (LDH) isozymes A (muscle) and B (heart) are best suited for pyruvate reduction and lactate oxidation, respectively. In mammals and columbid birds, a 3rd LDH-C isozyme is expressed in testis. In advanced teleost fish a 3rd LDH isozyme is found only in the eye, but in more primitive teleosts, if it is present, it has a generalized distribution. The evolutionary relationships among these 3rd forms of LDH isozymes have not been completely resolved.

We have cloned the cDNAs encoding LDH-A, LDH-B, and/or LDH-C isozymes from the human, mouse, rat, porcine, pigeon, alligator, lizard, turtle, *Xenopus*, and a nematode. We have determined the exon-intron organization of human LDH-A, LDH-B, and LDH-C genes, and the complete sequence of 12 851 nucleotides of the mouse LDH-A gene. The protein-coding sequences of the mammalian LDH-A, LDH-B, and LDH-C genes, as well as duck LDH-B gene, are interrupted by 6 introns at homologous positions. The expression of vertebrate LDH-A, LDH-B, and LDH-C genes is tissue specific and developmentally regulated. Expression of the mammalian LDH-A gene was also shown to be induced by cAMP and estrogen.

In collaboration with Dr. Wen-Hsiung Li, we analyzed the evolutionary relationships among vertebrate LDH isozymes. Contrary to the common opinion that the turtle lineage branched off before the divergence between the lizard/alligator and bird lineages, the turtle lineage was found clustered with either the alligator lineage or the alligator-bird clade, while the lizard lineage was found to have branched off before the divergence between the alligator/turtle and bird lineages. The pigeon testicular LDH-C gene was evidently duplicated from the LDH-B (heart) gene, so it is not orthologous to the mammalian testicular LDH-C genes, which appear to be derived from the LDH-A (muscle) gene.

Key words: Genes, Isozymes, Protein sequences, Phylogenetic tree.

INTRODUCTION

L-lactate dehydrogenase (LDH, EC1.1.1.27) catalyzes the interconversion of L-lactate and pyruvate with nicotinamide adenine dinucleotide (NAD⁺) as a coenzyme. The LDH isozyme system is one of the most extensively studied models used to investigate the origin and evolution of isozymes and the regulation of multigene families (Holmes 1972, Markert et al. 1975, Li 1990, Tsuji et al. 1994). In vertebrates, the LDH-A isozyme is best suited for pyruvate reduction in anaerobic tissues (muscle), whereas the LDH-B isozyme is superior for L-lactate oxidation in aerobic tissues (heart). In mammals and columbid birds, a third LDH isozyme is expressed in mature testes. However, only the LDH-A and LDH-B isozymes are present in other birds and in reptiles. In *Xenopus*, LDH isozymes are encoded by at least 3 LDH genes. In advanced teleost fishes, a 3rd LDH isozyme is found only in the eye, but in lower teleosts, if present, it has a generalized tissue distribution. The relationships among these LDH isozymes of vertebrates have not been completely resolved.

PROTEIN STRUCTURE-FUNCTION

In 1983, we elucidated the primary structure of testicular LDH-C isozymes from mouse and rat by amino acid sequencing (Pan et al. 1983). We subsequently determined the complete amino acid sequences of LDH-A (muscle), LDH-B (heart), and/or LDH-C (testis) from the human, mouse, rat, and porcine by sequencing both proteins and/or cDNAs (Li et al. 1985, Tsujibo et al. 1985, Sakai et al. 1987a,b, Hiraoka and Li 1990, Hiraoka et al. 1990, Wu and Li 1990, Tsuji et al. 1994). These results indicate that the loop region of mammalian testicular LDH-C sequences is quite different from those of somatic LDH-A and LDH-B isozymes. Since a major conformational change at the loop region occurs during catalysis (Eventoff et al. 1977), the amino acid changes at the loop region of the testicular LDH-C isozyme may well be reflected in its kinetic properties: broad substrate specificity, low turnover number, and high thermostability (Li et al. 1983a, Pan et al. 1983).

Recently, we have obtained complete amino acid sequences from the full-length cDNAs encoding LDH-A, LDH-B, and LDH-C of the pigeon, LDH-A and LDH-B of alligator, turtle and lizard (Mannen et al. 1996 1997); LDH-A, LDH-B, and LDH-C of *Xenopus* (Tsuji et al. 1994); and nematode LDH

(Tsoi and Li 1994). Rat liver single-stranded DNA binding protein was first identified as LDH-A isozyme (Williams et al. 1985, Li 1994). We have also shown that the low-salt eluting, single-stranded DNA-binding protein from mouse myeloma possesses LDH activity, and that human and bovine LDH-A isozymes are capable of binding single-stranded DNA (Sharief et al. 1986). The ϵ -crystalline lens protein from duck and alligator was unexpectedly found to be an active LDH-B isozyme (Wistow et al. 1987). The human LDH-B protein and centrosomal protein were reported to possess a common epitope (Gosti et al. 1992).

GENOMIC ORGANIZATION

In 1985, we described the exon-intron organization of human and mouse LDH-A genes (Chung et al. 1985, Li et al. 1985). In 1987, Fukasawa and Li reported the complete sequence of 12 851 nucleotides for the mouse LDH-A gene (Fukasawa and Li 1987). We subsequently characterized the exon organization of human LDH-B and LDH-C genes (Takano and Li 1989a,b). The protein-coding sequences of these mammalian LDH-A, LDH-B, and LDH-C genes, as well as the duck LDH-B gene, are interrupted by 6 introns at homologous positions. The locations of these 6 introns correspond to random-coil regions, or they are near ends of secondary structures on the surface of the monomeric LDH subunit (Li et al. 1985). It is of interest that the protein-coding sequence of nematode LDH was shown to be interrupted by only 2 introns corresponding to the 2nd and 6th of the 6 introns present in vertebrate LDH genes (Mannen and Li 1995).

The relationships between the exon organizations of these mammalian LDH-A, LDH-B, and LDH-C genes and the structural or functional domains of LDH-A, LDH-B, and LDH-C proteins are summarized in Figure 1 (Li et al. 1985). The DNA sequences coding for coenzyme-binding and catalytic domains are not separated by an intron. However, the 2 mononucleotide-binding subdomains are divided by an intron as predicted. The adenine and nicotinamide subdomains are further interrupted by introns. A comparison of the exon organization of the dinucleotide-binding domains from several NAD⁺-dependent dehydrogenase genes indicates that the mononucleotide-binding subdomain may have been formed by 3 basic exons coding for a common secondary structure of the $\alpha\beta$ unit (Fukasawa and Li 1987). The

“catalytic” domains may be divided into real catalysis and COOH-tail subdomains consisting of $\alpha\beta\beta\alpha$ units. The substrate-binding and negative ring of the catalytic subdomain are separated by an intron; the negative ring and carboxyl-tail are further interrupted by introns.

REGULATION OF EXPRESSION

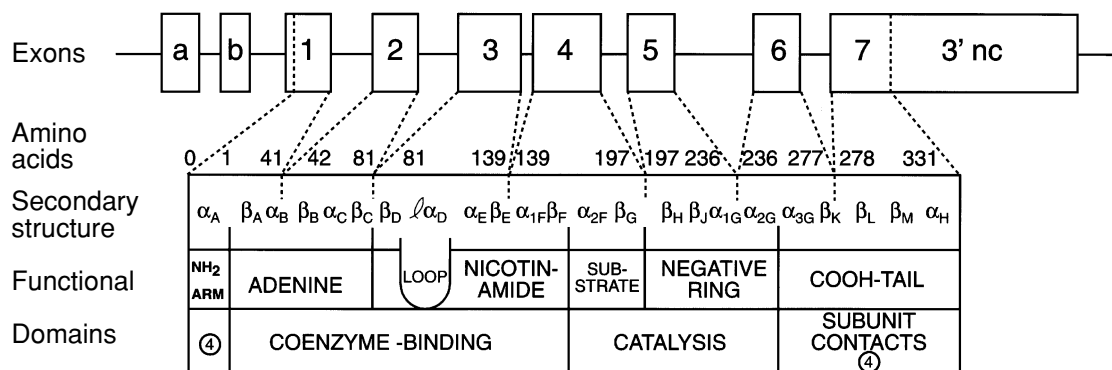
In 1989, we described the differential activity and synthesis of mouse LDH-A, LDH-B, and LDH-C isozymes during testicular development (Li et al. 1989). The testicular LDH-C isozyme was detected in isolated pre-leptotene and leptotene/zygotene spermatocytes prior to the mid-pachytene stage of meiosis. In addition, somatic-type LDH isozymes consisting primarily of LDH-B subunits were present in germ cells throughout spermatogenesis. This is in contrast to a previous report that the LDH-B subunit was not synthesized in germ cells (Hawtrey and Goldberg 1968). We have further investigated the expression during spermatogenesis of mouse LDH-A, LDH-B, and LDH-C genes using the cloned cDNAs as gene-specific probes. The results of Northern blot analysis and in situ hybridization studies confirmed that the expression of the LDH-C gene was detected prior to the pachytene stage of meiosis (Thomas et al. 1990). Further, expression of the LDH-A gene in testis appears to use a different promoter and/or alternative splicing from that of somatic tissues

(Hiraoka and Li 1990). As to the expression of the LDH gene during oogenesis, a steady-state level of LDH-B mRNA was found to accumulate throughout oogenesis, reaching unusually high levels in fully-grown oocytes; the LDH-B isozyme was also present and accumulated to very high levels in fully-grown mammalian oocytes and eggs (Roller et al. 1989).

We demonstrated the increased synthesis of LDH-A isozymes in uteri of immature mice treated with diethylstilbestrol (DES) (Li and Hou 1989). The promoter/regulatory region of the mouse LDH-A gene was fused with the bacterial chloramphenicol acetyltransferase (CAT) gene, and the expression of this fusion gene in Chinese hamster ovary cells was shown to be induced by estrogen (17β -estradiol/ DES) and/or cAMP (Hou and Li 1987). A palindromic sequence of 5'CTGACGTCAG3' present in the mouse LDH-A gene promoter was found to exhibit strong homology to the cAMP-responsive elements reported for other mammalian genes. An analysis of the putative promoter sequence of the human LDH-B gene indicates the absence of an apparent cAMP-responsive element. This is consistent with the observation that expression of the LDH-B gene is not induced by cAMP.

MOLECULAR EVOLUTION

In 1972, Holmes first proposed that vertebrate



Gene organization and structural-functional domains of LDH-A protein

Fig. 1. The relationship between exons of the LDH-A gene and structural-functional domains of the LDH-A protein. The protein-coding sequence of human and mouse LDH-A genes are interrupted by 6 introns as indicated (Li et al. 1985). The 5' noncoding (nc) sequence is encoded by either exon a or exon b and the first 24 nucleotides of exon 1, while the entire 3' noncoding region as well as the COOH-terminal 54 amino acids is encoded by exon 7. The exon organization of human LDH-B and LDH-C genes is essentially the same as that of the LDH-A gene (Takano and Li 1989a,b).

LDH-A, LDH-B, and LDH-C genes originated from an ancestral gene during the course of evolution. The vertebrate LDH-C gene was previously assumed (Markert et al. 1975) to be derived from the LDH-B gene on the basis of genetic linkage of pigeon LDH-B and LDH-C genes (Zinkham et al. 1969). In 1983, we first showed that amino acid sequences of the somatic LDH-A and LDH-B isozymes are more closely related to one another than either is to the mammalian testicular LDH-C isozyme (Li et al. 1983b). Together with W. M. Fitch, we reported that the mammalian testicular LDH-C subunit appears to have diverged very early, prior to the divergence of vertebrate LDH-A and LDH-B subunits (Tsuji et al. 1994).

In collaboration with W.-H. Li, we analyzed the evolutionary relationships among amino acid sequences of LDH isozymes from mammals, birds, reptiles, amphibians, fishes, and nematodes by using maximum parsimony (MP) and neighbor-joining (NJ) methods (Saitou and Nei 1987, Swofford 1993, Mannen et al. 1997). The MP tree and the NJ tree are largely consistent with each other. A most surprising inference from both the MP and NJ trees is that turtle LDH-A is clustered together with alligator LDH-A. Contrary to the general opinion that the turtle lineage branched off before the divergence between the lizard/alligator and bird lineages, the turtle lineage was found clustered with either the alligator lineage or the alligator-bird clade, while the lizard lineage was found to have branched off before the divergence between the alligator/turtle and bird lineages.

Both the MP and NJ trees strongly suggest that pigeon LDH-C (testis) is closely related to pigeon LDH-B (heart). This result, as well as the close linkage between the pigeon LDH-B and LDH-C loci (Zinkham et al. 1969), supports the hypothesis that pigeon LDH-C arose from a recent duplication of LDH-B (Holmes 1972, Markert et al. 1975), but it is not orthologous to mammalian testicular LDH-C. Both the MP and NJ trees indicate that mammalian LDH-C arose from a duplication of the mammalian LDH-A as previously suggested (Millan et al. 1987, Hendriks et al. 1988). This is also consistent with the fact that the LDH-A and LDH-C genes are located on human chromosome 11 and on mouse chromosome 7 (Li et al. 1988, Thomas et al. 1990).

Both the MP and NJ trees also strongly confirm that *Xenopus* LDH-A, LDH-B, and LDH-C belong to a cluster and that *Xenopus* LDH-C arose from a duplication of LDH-B (Tsuji et al. 1994). Both the MP and NJ trees further indicate that fish

LDH-C is a duplicate of LDH-B (Crawford et al. 1989, Quattro et al. 1993).

CONCLUSIONS

The protein-coding sequences of the mammalian LDH-A, LDH-B, and LDH-C genes, as well as the duck LDH-B gene, are interrupted by 6 introns at homologous positions. The expression of vertebrate LDH-A, LDH-B, and LDH-C genes are developmentally regulated and tissue specific. The expression of the mammalian LDH-A gene was also shown to be induced by cAMP and estrogen. The pigeon testicular LDH-C gene was evidently duplicated from LDH-B (heart) gene, so it is not orthologous to the mammalian testicular LDH-C genes, which seems to have been derived from the LDH-A (muscle) gene. The alligator and the turtle appear to be most closely related to each other, and the lizard appears to have branched off before the divergence between the alligator/turtle and the birds.

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脊椎動物乳酸去氫酶基因之構造、調控及進化

李水龍¹

在脊椎動物中 L- 乳酸去氫酶的異構酶 A (主要表現於肌肉) 及 B (主要表現於心臟) 各是研究乳酸還原及乳糖氧化最適當的蛋白。在哺乳類及哥倫比亞鳥中發現了第三種乳酸去氫酶, 其表現在睪丸中。在高等的真骨魚類中這種第三類乳酸去氫酶同時在肝及眼中發現, 但在低等的真骨魚類中, 此蛋白則廣泛的分布在體內。這三種乳酸去氫酶在演化上之間的關係並沒有完全的解決。

我及我的研究同仁已選殖了多種生物的乳酸去氫酶 A, B, C 三種異構酶, 包括人類, 鼠, 大白鼠, 豬, 鴿, 鱷魚, 蜥蜴, 龜, 非洲爪蟾及線蟲。並決定了人類乳酸去氫酶 A 基因的内-外子構造及定序了鼠的完整的乳酸去氫酶 A 基因之 12 851 核酸長度的序列。哺乳類動物及鴨子的乳酸去氫酶 A, B, C 基因的蛋白表現區域被 6 個内子分割在相似的位置。脊椎動物的乳酸去氫酶 A, B, C 表現為器官專一性且在細胞分化上受到調控。而哺乳類的乳酸去氫酶 A 的表現已證明受到 cAMP 及 estrogen 的激化。

在與李文雄博士的合作中, 我們分析了脊椎動物中各種乳酸去氫酶異構酶之間的演化關係。相對於傳統上相信龜的分支早於蜥蜴 / 鱷魚及鳥的分支, 我們發現龜的分支同於鱷魚或鱷魚 / 鳥的分化支, 而蜥蜴的分支則早於鱷魚 / 龜及鳥的分支。鴿子睪丸的乳酸脫氫酶 C 則明顯為複製於乳酸脫氫酶 B, 故其不同於哺乳類的乳酸脫氫酶 C, 因為在哺乳類中其衍生於乳酸去氫酶 A。

關鍵詞 : 乳酸去氫酶, 異構酶, 遺傳基因, 分子演化。

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