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Characterization of new medium-chain alcohol dehydrogenases adds resolution to duplications of the class I/III and the sub-class I genes

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ABSTRACT

Four additional variants of alcohol and aldehyde dehydrogenases have been purified and functionally characterized, and their primary structures have been determined. The results allow conclusions about the structural and evolutionary relationships within the large family of MDR alcohol dehydrogenases from characterizations of the pigeon (*Columba livia*) and dogfish (*Scyliorhinus canicula*) major liver alcohol dehydrogenases. The pigeon enzyme turns out to be of class I type and the dogfish enzyme of class III type. This result gives a third type of evidence, based on purifications and enzyme characterization in lower vertebrates, that the classical liver alcohol dehydrogenase originated by a gene duplication early in the evolution of vertebrates. It is discernable as the major liver form at about the level in-between cartilaginous and osseous fish. The results also show early divergence within the avian orders. Structures were determined by Edman degradations, making it appropriate to acknowledge the methodological contributions of Pehr Edman during the 65 years since his thesis at Karolinska Institutet, where also the present analyses were performed.

1. Introduction

Mammalian alcohol dehydrogenases (ADHs) of the MDR (medium-chain dehydrogenase/reductase) superfamily are well-known and constitute several classes with distinct structural and functional properties [1]. Class I is the traditional liver enzyme, originating from glutathione-dependent formaldehyde dehydrogenase [2] (identical to class III ADH [3]) by a gene duplication in early vertebrate evolution [4,5]. The timing has been traced to about 550 million years ago (MYA), before or at the origin of osseous fish, by the presence in fish of a class-mixed form [4] and by calculations from present species variability [4,5]. Additional duplications gave the other classes, and later ones also isozyme variability within the classes, mainly for class I [1]. The various forms take part in our defence against toxic alcohols and aldehydes. They also participate to different extents in specific metabolic and regulatory pathways, including in the important formation of retinoic acid [6] and elimination of ingested ethanol. The duplications and evolutionary changes in structure and function have been traced to such an extent that ADHs serve as model enzymes for successive evolution of novel forms [1,2,7].

Additional forms of ADH occur in nature, including in the related SDR (short-chain dehydrogenase/reductase) superfamily. The SDR and MDR superfamilies have been traced to very distant, successive origins during the emergence of the different domains of life, have in part a common building block but separate internal architectures, and also illustrate formation of metalloenzymes [7].

In spite of this knowledge, questions still remain and many details are lacking or unexplained. However, early questions on the relationships of the many prokaryotic, yeast and plant forms have been much resolved by the genome projects now in data banks. The MDR superfamily is presently known to have over 15,000 data bank entries, representing minimally 86 families, including at least 25 genes in the human, of which seven code for ADH

Abbreviations: MDR/SDR, medium-chain/short-chain dehydrogenases/reductases; ADH, alcohol dehydrogenase; MYA, million years ago.

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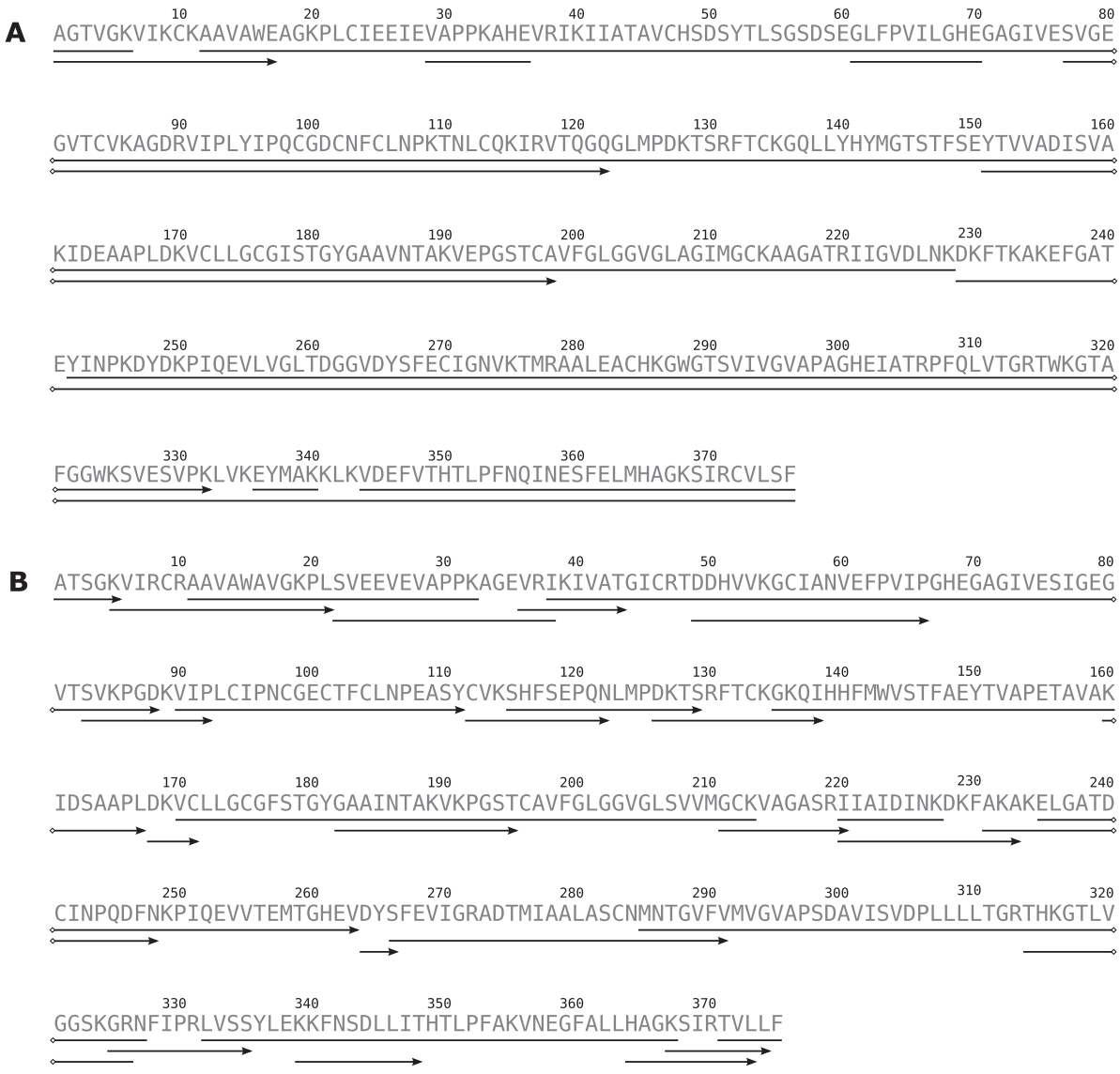

Fig. 1. Primary structures of the major liver alcohol dehydrogenase from (A) dogfish (*S. canicula*), and (B) feral pigeon (*C. livia*). Structures were determined by sequencer analyses of several sets of peptides for the regions shown by underlining.

[7,8]. The related SDR superfamily is even larger with over 47,000 entries, and minimally 300 families, representing at least 82 genes in the human [7,9], several involved in metabolic and hormonal conversions, activations and inactivations. Already in the early 2000 s, we decided to solve two remaining issues in the vertebrate MDR-ADH line, by isolation and analysis of two specific enzymes, the major liver ADH in shark and the one in a non-investigated avian order. The reason we wished to know these forms were:

- For the shark, because of our prediction that the major class I/III duplication originated at about the osseous fish stage (cf. above), where we found a class-mixed form [4], while below that stage (in Amphioxus, Ascidians [5] and octopus [10]) we found only class III. However, for the closest line below osseous fish, i.e. cartilaginous fish, an ADH had not been purified and functionally analyzed, although a partial sequence was reported in relation to an attempt to define the origin of tetrapods [11].
- For a species representing an avian order with non-analyzed ADH, because avian taxonomy needs further distinctions (cf. yearly revisions at http://www.birds.cornell.edu and previous comparisons involving other avian orders [12,13]). In addition, our studies of the bird ADHs from kiwi and ostrich (of the ancient avian order *Struthioniformes*) had revealed the presence of typical class I forms [14,15] as expected from our whole vertebrate assignment [1], while others had found in chicken an apparently different ADH classes [16].

We therefore isolated, class assigned, and determined the structure of liver ADH from dogfish from the west coast of Sweden, the closest source of cartilaginous shark available to us, to find a final proof for the major class duplication in vertebrates. Similarly, we isolated and analyzed liver ADH in feral pigeon of Stockholm, the closest source available to us of an avian order (*Columbiformes*) with non-analyzed ADH, to find further evidence on avian taxonomy. Additionally, we determined the primary structures of hamster testis ADH and aldehyde dehydrogenase (ALDH) to characterize also their divergences [17]. Results were conclusive but were not reported, except at a previous Carbonyl meeting, where we gave just the % difference values [18] without sequence information. We