## LDH isozyme profile of striated muscles and electrophoretically-typed single fibres from cane toad (*Bufo marinus*)

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In mammalian tissues (other than gonads), lactate dehydrogenase (LDH) is a tetrameric enzyme (Tsuji *et al*., 1994) produced by five random combinations of two different subunits, A and B [LDH1 ($B_4$)-lowest activity; LDH2 ($A_1B_3$); LDH3 ($A_2B_2$), LDH4 ($A_3B_1$); LDH5 ($A_4$)-highest activity], which are encoded by different genes (LDH-A and LDH-B). The expression of these genes and therefore the distribution of LDH isozymes ($LDH_{isoz}$) are tissue-specific. Using histochemically typed single fibre segments from rabbit skeletal muscles, Leberer & Pette (1984) found a predominance of B-type $LDH_{isoz}$ in type I fibres and of A-type $LDH_{isoz}$ in type II fibres, but found no difference in the $LDH_{isoz}$ profile between type IIA and IIB fibres. The authors concluded that the expression of the LDH subunits is not related to specific MHC isoforms ($MHC_i$). Recently we reported that rectus abdominis muscle of the cane toad contains a wide range of MHC-based fibre types that include pure twitch, hybrid twitch (*i.e.*, comprising one or several twitch $MHC_i$, respectively) and hybrid tonic-twitch fibres (Nguyen & Stephenson, 2002) of sizes that allow both $LDH_{isoz}$ and $MHC_i$ analyses. Currently there is no information on the number of $LDH_{isoz}$ in cane toad (*Bufo marinus*) skeletal muscles or on their distribution among different muscles and fibre types. In this study we aimed to address this cognitive gap using whole muscle homogenates and single muscle fibres from adult cane toads.

Male and female toads (79-149g) were killed by double pithing in accordance with procedures approved by Victoria University. Crude extracts from several skeletal [*rectus abdominis* ($n$ = 9), *hyoglossus* ($n$ = 4), *iliofibularis* ($n$ = 4), *semimembranosus* ($n$ = 6), and *sartorius* ($n$ = 5)] and cardiac ($n$ = 7) muscles of the toad and from mouse *soleus* muscle ($n$ = 4; used as a mammalian $LDH_{isoz}$ marker) were prepared in 0.9% NaCl and then further diluted in BPB-S solution [0.5 mg/ml bromphenol blue; 40% (w/v) sucrose; 0.1M Tris-HCl; pH 7.0] to a final concentration (w/v) of 0.45% (toad skeletal muscle), 1.2% (toad cardiac muscle), and 10.0% (mouse *soleus* muscle). Single fibre segments (1.8-85.3 nl), isolated from *rectus abdominis* muscles ($n$ = 6), were placed in 10.5 µl BPB-S solution and frozen at -84ºC for later analysis of $LDH_{isoz}$ and $MHC_i$. $LDH_{isoz}$ (in tissue extracts and single fibres) and $MHC_i$ (in single fibres) were resolved by non-denaturing polyacrylamide gel electrophoresis ($LDH_{isoz}$) and SDS-polyacrylamide gel electrophoresis ($MHC_i$) using the protocol of Leberer & Pette (1984), with modifications, and the method described by O'Connell *et al*. (2006), respectively. $LDH_{isoz}$ were visualized by an activity-based gel staining procedure using a system of coupled reactions which link lactate oxidation to reduction of nitroblue tetrazolium to formazan. $MHC_i$ bands were visualized by silver staining and quantified densitometrically (Molecular Dynamics). Each fibre segment was examined first for $LDH_{isoz}$ and then for $MHC_i$ composition.

Five $LDH_{isoz}$ were detected in both toad cardiac muscle and mouse *soleus* muscle, but their electrophoretic mobilities were different. These isozymes are presumably formed by the tetrameric combination of species-specific A and B subunits. With the exception of the toad *semimembranosus* muscle samples, which displayed the toad equivalent of mammalian LDH4 and LDH5 ($LDH5_{toad}$ and $LDH4_{toad}$) isozymes, all the toad skeletal muscle samples examined in this study displayed only one $LDH_{isoz}$, *viz.* $LDH5_{toad}$. The population of 100 single *rectus abdominis* fibres comprised 31 tonic-twitch hybrids, of which 14 fibres contained predominantly (> 80% $MHC_{total}$) the tonic $MHC_i$, and 69 pure and hybrid twitch fibres. Of the 14 predominantly tonic fibres, 13 fibres (93%) displayed only $LDH5_{toad}$, regardless of the identity or the number of the twitch $MHC_i$ co-expressed with the tonic isoform, and one fibre contained both $LDH5_{toad}$ and $LDH4_{toad}$. Similarly, 99% (68/69) of the pure and hybrid twitch fibres and 17 tonic-twitch hybrid fibres displayed only $LDH5_{toad}$, with one hybrid twitch fibre containing both $LDH5_{toad}$ and $LDH4_{toad}$. The $LDH_{isoz}$ composition of the two fibres containing both $LDH5_{toad}$ and $LDH4_{toad}$ was not unique to their MHC classification as other fibres of the same type showed only the $LDH5_{toad}$ isozyme. These results indicate that in cane toad skeletal muscles, like in rabbit skeletal muscles, there is no simple and tight relationship between the molecular forms of LDH (key enzyme in anaerobic metabolism) and those of MHC (molecular motor in muscle contraction).

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