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A preliminary account of the properties of recombinant human Glyoxylate reductase (GRHPR), LDHA and LDHB with glyoxylate, and their potential roles in its metabolism

K. Mdluli[a], M.P.S. Booth[b], R.L. Brady[b], G. Rumsby[c,*]

[a]Department of Immunology and Molecular Pathology, University College London, UK
[b]Department of Biochemistry, University of Bristol, London, UK
[c]Department Clinical Biochemistry, UCL Hospitals, 60 Whitfield St., London W1T 4EU, UK

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Abstract

Human lactate dehydrogenase (LDH) is thought to contribute to the oxidation of glyoxylate to oxalate and thus to the pathogenesis of disorders of endogenous oxalate overproduction. Glyoxylate reductase (GRHPR) has a potentially protective role metabolising glyoxylate to the less reactive glycolate. In this paper, the kinetic parameters of recombinant human LDHA, LDHB and GR have been compared with respect to their affinity for glyoxylate and related substrates. The K_m values and specificity constants (K_{cat}/K_M) of purified recombinant human LDHA, LDHB and GRHPR were determined for the reduction of glyoxylate and hydroxypyruvate. K_M values with glyoxylate were 29.3 mM for LDHA, 9.9 mM for LDHB and 1.0 mM for GRHPR. For the oxidation of glyoxylate, K_M values were 0.18 mM and 0.26 mM for LDHA and LDHB respectively with NAD^+ as cofactor. Overall, under the same reaction conditions, the specificity constants suggest there is a fine balance between the reduction and oxidation reactions of these substrates, suggesting that control is most likely dictated by the ambient concentrations of the respective intracellular cofactors. Neither LDHA nor LDHB utilised glycolate as substrate and NADPH was a poor cofactor with a relative activity less than 3% that of NADH. GRHPR had a higher affinity for NADPH than NADH (K_M 0.011 mM vs. 2.42 mM). The potential roles of LDH isoforms and GRHPR in oxalate synthesis are discussed.
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1. Introduction

Glyoxylate is a two carbon acid produced by the metabolism of glycolate and other unidentified sources. It is a highly reactive substance and at least two enzymes, alanine: glyoxylate aminotransferase (AGT) and glyoxylate reductase (GRHPR), are present in the hepatocyte which are able to metabolise glyoxylate to less toxic intermediates. Inherited deficiency of either of these enzymes leads to the metabolism of excess glyoxylate to oxalate with clinical sequelae characteristic of the primary hyperoxalurias (reviewed by [1]). Lactate dehydrogenase (LDH) is proposed to have a major role in oxalate production from glyoxylate (Fig. 1) particularly in situations of endogenous overproduction [2]. However, no kinetic data are available describing the relative affinity for glyoxylate of GRHPR and the different isoenzymes of LDH preventing any assessment of their relative roles in vivo.

LDH is a tetramer composed of LDHA and LDHB subunits and somatic cells contain heterotetramers formed from combinations of these subunits in different proportions. LDHA is particularly abundant in skeletal muscle and liver while LDHB is the major form in cardiac muscle. A third isoenzyme, LDHC, is expressed in spermatozoa and testis only [3]. The proteins are encoded by three different genes which map to different chromosomal locations, LDHA and LDHC to chromosome 11; LDHB to chromosome 12 [4,5].

* Corresponding author. Tel.: +44 20 7636 8333x2955; fax: +44 207 380 9584.
E-mail address: gill.rumsby@uclh.nhs.uk (G. Rumsby).

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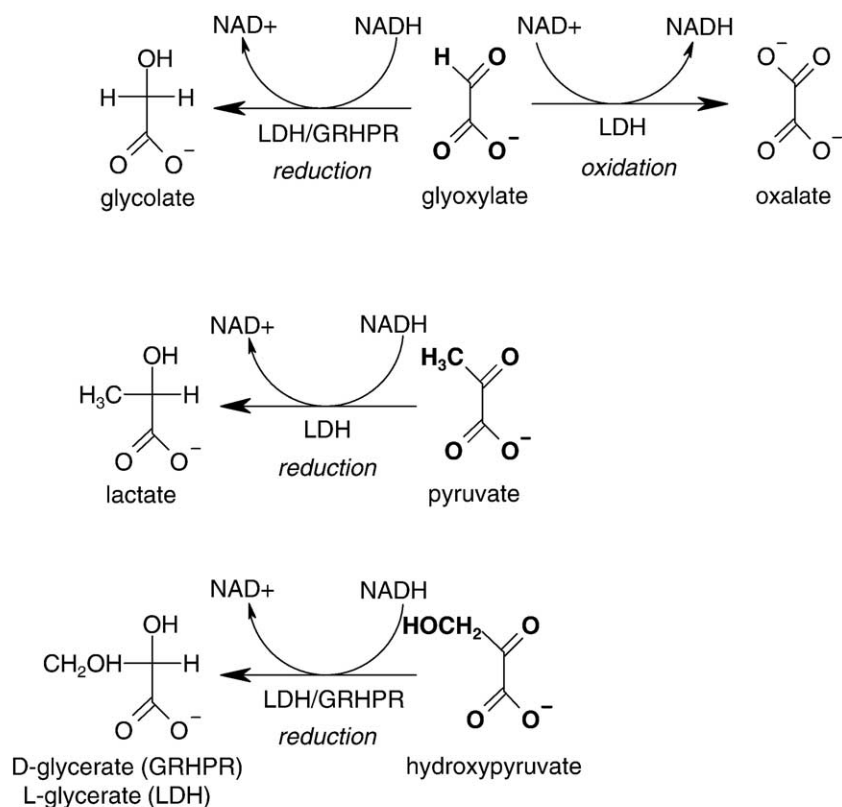

Fig. 1. Reactions catalysed by LDH and GRHPR. Schematic showing the reactions catalysed by LDH and GRHPR as measured in this study. Note that GRHPR, unlike LDH, can also utilise NADPH as a co-factor.

The three human LDHs have 84–89% sequence similarities and 69–75% amino acid identities [6]. However, in spite of this, they have been reported to have different substrate specificities and selective inhibitors of $LDHA_4$ and $LDHB_4$ have been described [7]. However, crystallographic analyses have shown the active sites of both forms to be identical, leading to the proposal that the distinctive catalytic properties results from electrostatic effects of residues peripheral to the active site [8]. The enzymes are regarded as cytosolic although there is some evidence that LDHA is also found in the mitochondria [9], where it may play a role in intracellular lactate metabolism. Others have disputed the mitochondrial localisation [10] and no mitochondrial targeting sequence has yet been identified. The role of LDH, and in particular the individual isoenzymes of human LDH, LDHA and LDHB, in glyoxylate metabolism has never been formally documented nor has its interaction with glyoxylate reductase (GRHPR).

Glyoxylate reductase is also primarily cytosolic with a minor component in the mitochondrion [11] with the bulk of enzyme activity located in the liver [12]. The enzyme also has hydroxypyruvate reductase and D-glycerate dehydrogenase activities (Fig. 1) although conditions for the latter appear to be unfavourable in the cell. Cloning and expression of the GRHPR gene [13,14] has enabled detailed kinetic analysis of the human enzyme, deficiency of which is known to underlie primary hyperoxaluria type 2 (PH2). In this disorder glyoxylate normally metabolised to glycolate by GRHPR is available for oxidation by LDH.

In this paper, we compare the kinetic properties of recombinant human LDHA, LDHB and GRHPR with glyoxylate to investigate the most likely primary route of disposal of cytosolic glyoxylate.

2. Materials and methods

All chemicals were of Analar grade and purchased from Sigma-Aldrich, Poole, Dorset, UK unless otherwise stated. Expression plasmid pEZZ-18 was purchased from Amersham Biosciences (Chalfont St. Giles, UK) and JM109 competent cells from Stratagene (Cambridge). LDHB cDNA Image Clone ID 2961445 was obtained from I.M.A.G.E. Consortium (http://menu.hgmp.mrc.ac.uk/) and human LDHA cloned into pKK223-3 vector as previously described [15]. Custom-made oligonucleotides were obtained from Genosys (Genosys Biotechnologies, Pampisford) and Taq polymerase from Promega (Southampton).

2.1. Expression and purification of recombinant human LDHA

Recombinant human LDHA in the pKK223-3 vector was expressed without induction as previously described [15]. Crude enzyme extract was prepared by suspending cells in 20 mM phosphate buffer containing Bugbuster™ (Novagen, Madison, USA) protein extraction reagent (10%v/v). The

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