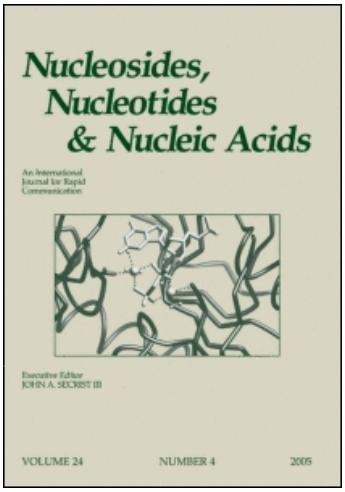

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Tissue Specific Distribution of Pyrimidine Deoxynucleoside Salvage Enzymes Shed Light on the Mechanism of Mitochondrial DNA Depletion

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Online publication date: 11 June 2010

To cite this Article Wang, L. and Eriksson, S.(2010) 'Tissue Specific Distribution of Pyrimidine Deoxynucleoside Salvage Enzymes Shed Light on the Mechanism of Mitochondrial DNA Depletion', Nucleosides, Nucleotides and Nucleic Acids, 29: 4, 400 — 403
To link to this Article: DOI: 10.1080/15257771003741042
URL: http://dx.doi.org/10.1080/15257771003741042

Nucleosides, Nucleotides and Nucleic Acids, 29:400–403, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257771003741042

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TISSUE SPECIFIC DISTRIBUTION OF PYRIMIDINE DEOXYNUCLEOSIDE SALVAGE ENZYMES SHED LIGHT ON THE MECHANISM OF MITOCHONDRIAL DNA DEPLETION

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□ *Deficiency in thymidine kinase 2 (TK2) activity due to genetic alterations caused tissue specific mitochondrial DNA (mtDNA) depletion syndrome with symptoms resembling these of AIDS patients treated with nucleoside analogues. Mechanisms behind this mitochondrial effects is still not well understood. With rat as a model we isolated mitochondrial and cytosolic fractions from major organs and studied enzymes involved in thymidine (dT) and deoxycytidine (dC) phosphorylation by using ionic exchange column chromatography. A cytosolic form of TK2 was identified in all tested tissues in addition to mitochondrial TK2. TK1 was detected in liver and spleen cytosolic extracts while dCK was found in liver, spleen and lung cytosolic extracts. Thus, the nature of dT and dC salvage enzymes in each tissue type was determined. In most tissues TK2 is the only salvage enzyme present except liver and spleen. These results may help to explain the mechanisms of mitochondrial toxicity of antiviral nucleoside analogues and mtDNA depletion caused by TK2 deficiency.*

Keywords Thymidine kinase 2; rat tissue; mitochondrial; cytosolic; mitochondrial DNA depletion

INTRODUCTION

Mitochondrial DNA (mtDNA) depletion syndrome is a mitochondrial disorder characterized by quantitative reduction in mtDNA molecules in a tissue specific manner, for example, predominantly in liver or muscle. Recently, deficiency in thymidine kinase 2 (TK2) activity or alteration of TK2 specificities, due to point mutations, insertion and deletion of the TK2 gene, in humans has been associated with tissue specific mitochondrial DNA depletion that led to severe myopathy.[1] The underlining mechanism of this mtDNA disorder has been suggested to be imbalanced mitochondrial deoxynucleotide pools caused by the TK2 deficiency.[2,3]

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Using mitochondrial and cytosolic extracts prepared from rat tissues we studied enzymes in pyrimidine deoxynucleoside salvage. A cytosolic form of TK2 was identified in addition to mitochondrial TK2. The distribution of dT and dC salvage enzymes is tissue specific, which may explain why TK2 deficiency activity led to mtDNA depletion.

MATERIAL AND METHODS

Radioactive dT ([methyl-^{3}H]-dT, 20 Ci/mmol) was from Perkin Elmer Srerige AB (Upplands Vasby, Sweden) and dC ([5–^{3}H]-dC, 27 Ci/mmol) was from GE Healthcare. Deoxynucleoside kinase activity was determined using either ^{3}H-dT, or ^{3}H-dC as substrate as previously described.[4] Mitochondria and cytosol fractions were prepared from fresh rat tissues (300–350 g, Sprague Dawley, Scanbur B&K, Sollentuna, Sweden) by differential centrifugation.[5] Western blot analyses using antibodies against cytochrome c and cytochrome c oxidase subunit 4 (Cox 4) were carried with both mitochondrial and cytosolic proteins from each preparation, and cytochrome c and Cox 4 were detected only in the mitochondrial preparations but not in the cytosol preparations, which demonstrated that the cytosol preparations are free from mitochondria (or mitochondrial proteins) contamination. Mitochondrial proteins were extracted by addition of 0.5% NP-40 and sonication in. Protein concentration was determined by the Bio-Rad protein assay using BSA as standard. Mitochondrial or cytosolic protein extracts were separated on anion exchange column (DEAE fast flow, GE Healthcare, Uppsala Sweden column size 1 ml) and elution was done essentially as described.[6] Flow through from the DEAE column was collected and applied to a cationic exchange column (CM-sepharose, GE Healthcare, column size 1 ml) and eluted with linear gradient 0–0.6 M KCl. Fractions (0.5 ml) were collected and assayed.

RESULTS AND DISCUSSION

Tissue and Subcellular Distribution of TK1, TK2, and dCK in Rat Tissues

Both dT and dC kinase activities were detected in crude mitochondrial and cytosolic extracts. To further distinguish the specific enzyme responsible for the activities detected, mitochondrial respective cytosolic proteins prepared from liver were separated by anion exchange column (DEAE) chromatography. Both dT and dC kinase activities co-eluted at salt concentration ~0.15 M (Figure 1A), demonstrating the presence of TK2 in the mitochondria.[6] dC kinase activity was detected in the fractions eluted with ~0.3 M salt and there was no dT kinase activity detected upon DEAE chromatography, suggesting the presence of dCK (Figure 1A). Flow through from DEAE

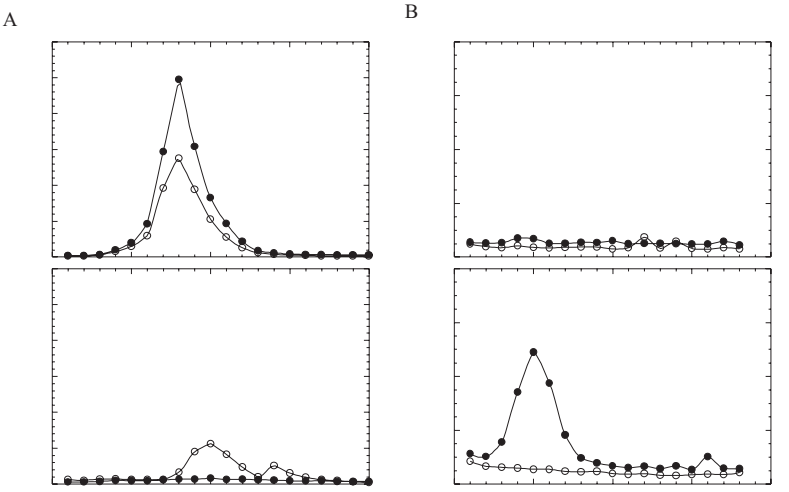

FIGURE 1 Ionic exchange chromationgraphy of mitochondrial and cytosolic extracts from rat liver. A) DEAE chromatograms; B) CM-sepharose chromatograms. Fractions were collected and assayed with dThd (•) and dCyd (○) as substrates.

chromatography of cytosolic extracts was collected and applied to a cationic exchange column (CM-sepharose) and eluted. dT kinase activity was clearly detected in the fractions eluted with salt concentration ~0.15 M and no dC kinase activity was present, this represents TK1 (Figure 1B). Flow through from DEAE chromatography of mitochondrial extracts were also collected and applied to a CM-sepharose column and there was no dT/dC kinase activity detected in any of the eluted fractions (Figure 1B).

DEAE and CM-sepharose chromatography were also performed with cytosolic proteins prepared from heart, spleen, lung, kidney, skeletal muscle, and brain tissues (data not shown). TK2 activity was detected in brain, heart, lung, kidney, skeletal muscle, and spleen, while dCK activity was detected in spleen and lung and TK1 activity was detected in spleen (Table 1). In this way we have clarified the nature of dT and dC salvage enzymes in rat tissues.

TABLE 1 Tissue distribution of pyrimidine deoxynucleoside salvage enzymes

Tissue	Mitochondria	Cytosol
Liver	TK2	TK1, dCK
Heart	TK2	TK2
Brain	TK2	TK2
Kidney	TK2	TK2
Spleen	TK2	TK1, TK2, dCK
Lung	TK2	TK2, dCK
Skeletal muscle	TK2	TK2

Tissue Specific Distribution of Mitochondrial TK2 Activity

The mitochondrial TK2 activities varied a lot between tissues; there was over 100-fold difference in TK2 activity between the lowest in skeletal muscle mitochondria and the highest in spleen mitochondria. The fold difference was in the order of skeletal muscle (1-), heart (5-), kidney (20-), liver (40-), brain (90-), and spleen (122-), respectively.

Cytosolic TK1, TK2, and dCK Activities

In liver cytosol, TK1 activity was present at low level (0.04 pmol/min/mg) and is the only dThd kinase found. dCK was also present in liver cytosol at a very low level (0.06 pmol/min/mg). In spleen cytosolic TK (TK1 + TK2) activity is high due to high TK1 level. dCK was present at high level in spleen. In the cytosol of heart, brain, lung, skeletal muscle, and kidney TK2 activity levels were low (0.2–0.8 pmol/min/mg) and skeletal muscle contained the lowest level of cytosolic TK2 (0.07 pmol/min/mg).

This work represented accurate measurements of TK2 levels in major mammalian organs and clarified the nature of cytosolic dT and dC salvage enzymes in post-mitotic tissues. The tissue specific distribution of TK2 correlated to the clinical observation that skeletal muscle, has the lowest TK2 activity, and is the tissue that is mostly affected in chemotherapy with pyrimidine nucleoside analogues which are TK2 substrates and/or inhibitors. Furthermore, these results may also explain why TK2 deficiency due to genetic alteration mainly affected muscle tissues.

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