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Short sequence-paper

The cDNA sequence of proton-pumping nicotinamide nucleotide transhydrogenase from man and mouse

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Abstract

cDNA clones for the human and mouse nicotinamide nucleotide transhydrogenases have been isolated and their sequences have been determined. Multiple alignments show that the functional proteins are encoded by single mRNAs. The deduced amino acid sequences are approximately 95% identical for the previously known bovine, and the human and mouse proteins. The major variable region is located in the presequence. It is proposed that all mammalian transhydrogenases have a similar structure.

Keywords: Transhydrogenase; Proton pumping; NAD; NADP; cDNA; (Human); (Mouse)

Nicotinamide nucleotide transhydrogenase (TH) (EC 1.6.1.1) is a redox-driven proton pump which catalyzes the reversible reduction of $NADP^+$ by NADH according to the reaction

$$H^+_{out} + NADP^+ + NADH \rightleftharpoons H^+_{in} + NAD^+ + NADPH$$

where protons are pumped from the cytosol or periplasmic space (out) to the matrix or the cytosol (in) in mitochondria and certain bacteria, respectively. Transhydrogenases are found in most micoorganisms and all mammalian tissues investigated, and the transhydrogenase gene is also expressed in man [1]. The enzymes from bovine, *E. coli* and *Rhodospirillum rubrum* have been characterized extensively in their detergent-dispersed pure states as well as in reconstituted phospholipid vesicles [1]. The genes of these and several other transhydrogenases have been cloned and their cDNA/DNA sequenced [1], but only the *E. coli* [2,3] and *R. rubrum* [4] transhydrogenase genes have so far been overexpressed. Except for the bovine enzyme, which is composed of a homodimeric active form of a single subunit, other transhydrogenases are composed of two or three subunits also arranged as homodimeric active forms [1]. However, among the mammalian enzymes only the bovine transhydrogenase has been completely characterized with respect to amino acid sequence [5]. It is therefore still uncertain whether the bovine enzyme is a general, single subunit, representative of mammalian transhydrogenases. In order to resolve this problem and to open the way for studies of transhydrogenase gene regulation in mammals, we have cloned the genes for the human and mouse transhydrogenases.

The cloning strategies were conducted essentially as described by Maniatis et al. [6]. Commercial cDNA libraries in the Lambda ZAP vector (936208 human male heart and 935302 mouse female liver, Stratagene, USA), were screened using cDNA from the bovine transhydrogenase as probe (construct pUGO4; Ref. [7]). The probe was ^{32}P-labeled with the Mega-Prime kit and ^{32}P-dATP obtained from Amersham (UK). Positive clones were identified by autoradiography. The libraries were screened as described by Stratagene. Several positive clones were isolated and clones with large inserts were chosen for sequencing. They were excised as pBluescript and single-stranded DNA rescues were performed as described by Stratagene. Clones were sequenced both as plasmid preparations (QIAGEN, USA) and as single-strand rescues. DNA sequencing was carried out by the dideoxynucleotide method of Sanger et al. [8] on an ALF (Automated Laser Fluorescent) DNA sequencer (Pharmacia, Sweden), using the AutoRead kit (Pharmacia, Sweden) and the Long Ranger, Hydrolink, gel (AT Biochem, USA). Both M13 reverse primer and internal fluorescein-labelled sequencing

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primers obtained from Scandinavian Gene Synthesis (Linköping, Sweden) were used.

The mouse sequence was covered by two overlapping clones and the human by three overlapping clones; all of these clones were completely sequenced. The sequences have been submitted to GenBank/EMBL: accession numbers Z49204 (mouse) and Z50101 (human).

As shown in Fig. 1 the human and mouse transhydrogenase cDNA sequences were translated and aligned to the bovine sequence with the GCG program PileUp [9]. (Fig. 1). The human and mouse proteins are identical to the bovine protein in length [5] and all have a 43 amino acid long pre-sequence [10]. Thus, all three proteins are single gene products. In contrast, the bacterial enzymes are 2–3 gene products. Like the mammalian enzymes, the parasite transhydrogenase genes are also single genes but the order of transcription of the two halves of the genes is reversed [1]. The human and bovine proteins are 97% identical, whereas the mouse protein is approximately 94% identical to both the human and the bovine proteins. Differences are most abundant in the region 400–450 aa counted from the N-terminal of the mature protein. This hyper-variable region is located in the first predicted transmembrane α-helix including the preceding and succeeding loops, according to a prediction model made for the bovine enzyme by Holmberg et al. [7].

The pre-sequences are less conserved, between 69% and 81% identical and mainly hydrophobic (Fig. 1). Alignment of the presequences as a helical wheel shows that there are four semi/conserved positive amino acids distributed mainly on one half of the wheel (Fig. 2). This pre-sequence probably directs the premature protein to the

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       1                  Presequence                                                                                      100
Bovth  MANLLKTVVT GCSCPFLSNL GSCKVLPGKK NFLRTFHTHR ILWCSAPVKP GIPYKQLTVG VPKEIFQNEK RVALSPAGVQ ALVKQGFNVV VESGAGEASK
Mouth  MAHLLKTVVA GCSCPFLSNL GSSKVLPGKR DFVRMLRTHQ ALWCKSPVKP GIPYKQLTVG VPKEIFQNEK RVALSPAGVQ ALVKQGFNVV VESGAGEASK
Humth  MANLLKTVVT GCSCPLLSNL GSCKGLRVKK DFLRTFYTHQ ELWCKAPVKP GIPYKQLTVG VPKEIFQNEK RVALSPAGVQ NLVKQGFNVV VESGAGEASK

       101                                                                                                         200
Bovth  FSDDHYRAAG AQIQGAKEVL ASDLVVKVRA PMLNPTLGVH EADLLKTSGT LISFIYPAQN PDLLNKLSKR KTTVLAMDQV PRVTIAQGYD ALSSMANIAG
Mouth  FPDDLYRAAG AQIQGMKEVL ASDLVVKVRA PMVNPTLGAH EADFLKPSGT LISFIYPAQN PDLLNKLSER KTTVLAMDQV PRVTIAQGYD ALSSMANISG
Humth  FSDDHYRVAG AQIQGAKEVL ASDLVVKVRA PMVNPTLGVH EADLLKTSGT LISFIYPAQN PELLNKLSQR KTTVLTMDQV PRVTIAQGYD ALSSMANIAG

       201                                          NAD(H)                                                         300
Bovth  YKAVVLAANH FGRFFTGQIT AAGKVPPAKI LIVGGGVAGL ASAGAAKSMG AIVRGFDTRA AALEQFKSLG AEPLEVDLKE SGEGQGGYAK EMSKEFIEAE
Mouth  YKAVVLAANH FGRFFTGQIT AAGKVPPAKI LIVGGGVAGL ASAGAAKSMG AVVRGFDTRA AALEQFKSLG AEPLEVDLKE SGEGQGGYAK EMSKEFIEAE
Humth  YKAVVLAANH FGRFFTGQIT AAGKVPPAKI LIVGGGVAGL ASAGAAKSMG AIVRGFDTRA AALEQFKSLG AEPLEVDLKE SGEGQGGYAK EMSKEFIEAE

       301                                                                                                         400
Bovth  MKLFAQQCKE VDILISTALI PGKKAPILFN KEMIESMKEG SVVVDLAAEA GGNFETTKPG ELYVHKGITH IGYTDLPSRM ATQASTLYSN NITKLLKAIS
Mouth  MKLFAQQCKE VDILISTALI PGKKAPVLFS KEMIESMKEG SVVVDLAAEA GGNFETTKPG ELYVHKGITH IGYTDLPSRM ATQASTLYSN NITKLLKAIS
Humth  MKLFAQQCKE VDILISTALI PGKKAPVLFN KEMIESMKEG SVVVDLAAEA GGNFETTKPG ELYIHKGITH IGYTDLPSRM ATQASXLYSN NITKLLKAIS

       401                                                                              1                          500
Bovth  PDKDNFYFEV KDDFDFGTMG HVIRGTVVMK DGQVIFPAPT PKNIPQGAPV KQKTVAELEA EKAATITPFR KTMTSASVYT AGLTGILGLG IAAPNLAFSQ
Mouth  PDKDNFHFEV KDDFDFGTMS HVIRGTVVMK DGKVIFPAPT PKNIPEEAPV KPKTVAELEA EKAGTVSMYT KTLTTASVYS AGLTGMLGLG IVAPNVAFSQ
Humth  PDKDNFYFDV KDDFDFGTMG HVIRGTVVMK DGKVIFPAPT PKNIPQGAPV KQKTVAELEA EKAATITPFR KTMSTASAYT AGLTGILGLG IAAPNLAFSQ

       501     2                       3                    4                                                      600
Bovth  MVTTFGLAGI VGYHTVWGVT PALHSPLMSV TNAISGLTAV GGLVLMGGHL YPSTTSQGLA ALATFISSVN IAGGFLVTQR MLDMFKRPTD PPEYNYLYLL
Mouth  MVTTFGLAGI IGYHTVWGVT PALHSPLMSV TNAISGLTAV GGLALMGGHF YPSTTSQSLA ALATFISSVN IAGGFLVTQR MLDMFKRPTD PPEYNYLYLL
Humth  MVTTFGLAGI VGYHTVWGVT PALHSPLMSV TNAISGLTAV GGLALMGGHL YPSTTSQGLA ALAAFISSVN IAGGFLVTQR MLDMFKRPTD PPEYNYLYLL

       601                                                       5                     6                           700
Bovth  PAGTFVGGYL ASLYSGYNIE QIMYLGSGLC CVGALAGLST QGTARLGNAL GMIGVAGGLA ATLGGLKPCP ELLAQMSGAM ALGGTIGLTI AKRIQISDLP
Mouth  PGGTFVGGYL AALYGGYNIE EIMYLGSGLC CVGALGGLST QGTARLGNAL GMIGVAGGLA ATLGGLKPDP QLLAQMSGAM AMGGTIGLTI AKRIQISDLP
Humth  PAGTFVGGYL AALYSGYNIE QIMYLGSGLC CVGALAGLST QGTARLGNAL GMIGVAGGLA ATLGVLKPGP ELLAQMSGAM ALGGTIGLTI AKRIQISDLP

       701        7                           8                                                                    800
Bovth  QLVAAFHSLV GLAAVLTCIA EYIIEYPHFA TDAAANLTKI VAYLGTYIGG VTFSGSLVAY GKLQGILKSA PLLLPGRHLL NAGLLAGSVG GIIPFMMDPS
Mouth  QLVAAFHSLV GLAAVLTCMA EYIVEYPHFA MDATSNFTKI VAYLGTYIGG VTFSGSLVAY GKLQGILKSA PLLLLGRHAL NAGLLAASVG GIIPFMADPS
Humth  QLVAAFHSLV GLAAVLTCIA EYIIEYPHFA TDAAANLTKI VAYLGTYIGG VTFSGSLIAY GKLQGLLKSA PLLLPGRHLL NAGLLAASVG GIIPFMVDPS

       801          9                                               10                                             900
Bovth  FTTGITCLGS VSALSAVMGV TLTAAIGGAD MPVVITVLNS YSGWALCAEG FLLNNNLLTI VGALIGSSGA ILSYIMCVAM NRSLANVILG GYGTTSTAGG
Mouth  FTTGITCLGS VSALSTLMGV TLTAAIGGAD MPVVITVLNS YSGWALCAEG FLLNNNLLTI VGALIGSSGA ILSYIMCVAM NRSLANVILG GYGTTSTAGG
Humth  FTTGITCLGA VSALSAVMGV TLTPAIGGAD MPVVITVLNS YSGWALCAEG FLLNNNLLTI VGALIGSSGA ILSYIMCVAM NRSLANVILG GYGTTSTAGG

       901                                          NADP(H)                                                        1000
Bovth  KPMEISGTHT EINLDNAIDM IREANSIIIT PGYGLCAAKA QYPIADLVKM LSEQGKKVRF GIHPVAGRMP GQLNVLLAEA GVPYDIVLEM DEINHDFPDT
Mouth  KPMEISGTHT EINLDNAVEM IREANSIVIT PGYGLCAAKA QYPIADLVKM LTEQGKKVRF GIHPVAGRMP GQLNVLLAEA GVPYDIVLEM DEINSDFPDT
Humth  KPMEISGTHT EINLDNAIDM IREANSIIIT PGYGLCAAKA QYPIADLVKM LTEQGKKVRF GIHPVAGRMP GQLNVLLAEA GVPYDIVLEM DEINHDFPDT

       1001
Bovth  DLVLVIGAND TVNSAAQEDP NSIIAGMPVL EVWKSKQVIV MKRSLGVGYA AVDNPIFYKP NTAMLLGDAK KTCDALQAKV RESYQK*
Mouth  DLVLVIGAND TVNSAAQEDP NSIIAGMPVL EVWKSKQVIV MKRSLGVGYA AVDNPIFYKP NTAMLLGDAK KTCDALQAKV RESYQK*
Humth  DLVLVIGAND TVNSAAQEDP NSIIAGMPVL EVWKSKQVIV MKRSLGVGYA AVDNPIFYKP NTAMLLGDAK KTCDALQAKV RESYQK*
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Fig. 1. Multiple alignment of the primary sequences for the bovine (Bovth), mouse (Mouth) and human (Humth) transhydrogenases. Predicted membrane spanning α-helices are shown and numbered according to Holmberg et al. [7]. The proposed two substrate binding sites for NAD(H) and NADP(H), respectively, are also marked. Non-identical amino acids are bold. Stars denote stop codons.

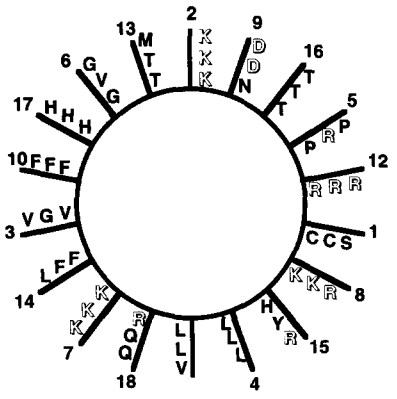

Fig. 2. Multiple alignment of the bovine, mouse and human transhydrogenase pre-sequences (aa 23–40) as a helical wheel. Uncharged residues are in plain text and charged residues are shadowed.

mitochondrion, where the charged surface may interact with a mitochondrial membrane receptor.

Based on the high similarity between the bovine, human and mouse transhydrogenases it is concluded that all mammalian transhydrogenases have a similar structure. The establishment of the human transhydrogenase sequence will enable more detailed studies on the regulation of the enzyme at the gene level and a possible correlation between expression and disease.

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