

RESEARCH COMMUNICATION

Molecular cloning and deduced amino acid sequences of the γ -subunits of rat and monkey NAD⁺-isocitrate dehydrogenases

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A 600 bp cDNA fragment encoding part of the γ -subunit of pig heart NAD⁺-isocitrate dehydrogenase (ICDH γ) was amplified by PCR using redundant oligonucleotide primers based on partial peptide sequence data [Huang and Colman (1990) *Biochemistry* 29, 8266–8273]. This PCR fragment was then used as a probe to isolate clones encoding the complete mature forms of the γ -subunit from rat epididymis and monkey testis cDNA

libraries. Comparison of the deduced amino acid sequences of the rat and monkey subunits and the partial sequence of the pig heart enzyme revealed a remarkably high level of sequence identity. The relationship between the deduced amino acid sequences of the NAD⁺-ICDH γ -subunits and those of non-mammalian NAD⁺- and NADP⁺-ICDH subunits is discussed.

INTRODUCTION

NAD⁺-isocitrate dehydrogenase (NAD⁺-ICDH) is one of three enzymes which catalyse the oxidation of *threo*-D₃-isocitrate to oxoglutarate and carbon dioxide in eukaryotic cells, the other two enzymes being the cytoplasmic and mitochondrial forms of NADP⁺-isocitrate dehydrogenase (NADP⁺-ICDH). NAD⁺-ICDH is located within mitochondria and is a component of the citrate cycle. Although mitochondria from many sources contain NADP⁺-ICDH activities which can greatly exceed that of NAD⁺-ICDH, NAD⁺-ICDH appears to be the sole important source of reducing equivalents for the respiratory chain (Plaut, 1970; Colman, 1975; Haselbeck and McAlister-Henn, 1993).

NAD⁺-ICDH has complex regulatory properties which are consistent with it contributing to the control of the citrate cycle. In particular, it exhibits sigmoidal kinetics with respect to isocitrate and is activated by increasing ADP/ATP ratios (which decrease the K_m for isocitrate) (Plaut, 1970; Gabriel et al., 1985; Rutter and Denton, 1989a). In addition, the enzyme from vertebrates, but not from invertebrates, plants or yeast, is activated by Ca²⁺ (Denton et al., 1978; McCormack and Denton, 1981; Rutter and Denton, 1989a; B. J. Nichols and R. M. Denton, unpublished work). Pyruvate dehydrogenase phosphatase and oxoglutarate dehydrogenase from vertebrates are also activated by Ca²⁺. Parallel activation of the three dehydrogenases is an important means whereby ATP production is accelerated in cells stimulated by hormones or other extracellular agents which act through increases in cell Ca²⁺ (McCormack et al., 1990; Denton and McCormack, 1990).

Previously, the only available cloned NAD⁺-ICDH subunits were from the yeast *Saccharomyces cerevisiae* (Cupp and McAlister-Henn, 1991, 1992). NAD⁺-ICDH from *S. cerevisiae* is an octamer composed of two similar subunits of M_r close to 40000. In contrast, NAD⁺-ICDH from pig and ox heart (and presumably other mammalian sources) has a more complex range of oligomeric structures based on three subunits, α , β and γ , each of M_r close to 40000, in the apparent ratio of 2:1:1 (Ramachandran and Colman, 1980; Ehrlich et al., 1981). The

contribution of each subunit to the catalytic and regulatory properties of the mammalian holoenzyme is unknown. All three subunits are labelled by incubation with the isocitrate analogue 3-ene-2-oxoglutarate (Bednar and Colman, 1982), but a single peptide exclusive to the γ -subunit was covalently modified by an alternative analogue, 3-bromo-2-oxoglutarate (Saha et al., 1989). Binding studies suggest that there is only one Ca²⁺-binding site per $\alpha_2\beta\gamma$ unit (Rutter and Denton, 1989b).

In this paper, we report for the first time the molecular cloning and deduced amino acid sequences of NAD⁺-ICDH γ subunits from mammalian sources. Comparisons of these sequences and those of yeast NAD⁺-ICDH and *Escherichia coli* NADP⁺-ICDH suggest that all share a common ancestry.

MATERIALS AND METHODS

Materials

Restriction endonucleases, T4 DNA ligase, T4 polynucleotide kinase, avian myeloblastosis virus (AMV) reverse transcriptase, deoxynucleotides and *Sma*I-cut, dephosphorylated pUC18 DNA were from Pharmacia, Milton Keynes, U.K. *Taq* polymerase was from Boehringer Mannheim, Lewes, E. Sussex, U.K. Random 14-mer DNA labelling kit was obtained from Du Pont-NEN, Stevenage, Herts., U.K. Nitrocellulose filters and micro-dialysis membranes were from Schleicher and Schull and Millipore respectively. All other chemicals were of AnalaR grade or the purest grade available. Construction and storage of rat (*Rattus norvegicus*) epididymal and monkey (*Macaca fascicularis*) testis cDNA libraries have been described previously (Perry et al., 1992a). Fresh heart tissue was obtained from a 2-month-old pig (*Sus scrofa*).

PCR amplification of pig heart ICDH γ cDNA

Total pig heart RNA (5 μ g) was used in oligo(dT)₁₂₋₁₈-primed cDNA synthesis catalysed by AMV reverse transcriptase. PCR amplification of the resultant cDNA was then carried out using primers based on published pig NAD⁺-ICDH peptide sequences

Abbreviations used: ICDH, isocitrate dehydrogenase; ICDH γ , γ -subunit of isocitrate dehydrogenase; AMV, avian myeloblastosis virus.

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The nucleotide sequences of rat (*Rattus norvegicus*) and monkey (*Macaca fascicularis*) NAD⁺-ICDH γ -subunit cDNAs will appear in the EMBL Nucleotide Sequence Database under the accession numbers X74125 and X74124 respectively.

(Huang and Colman, 1990; M. Leake and R. M. Denton, unpublished work). In total, eleven primers with redundancies varying from 24- to 1026-fold were used. The relative orientation of members of each primer pair was based on alignment of the available pig NAD⁺-ICDH peptide sequence with that deduced for both subunits of yeast NAD⁺-ICDH. All possible primer combinations were employed, at a variety of annealing temperatures and concentrations of cDNA, primers and dNTPs. The successful primers were: (1) 5'-TAYGCBAAYGTBATHC-AYTG-3'; (2) 5'-TTBARRTGRTCBARCATCAT-3'; and (3) 5'-ARRTGRTCNARCATCATRCA-3' (see Figure 2). Primers 2 and 3 were based on overlapping regions of the same peptide fragment, so that two near-identical PCR products were produced, one being four nucleotides longer at the 3' end (see above and Figure 2). Successful PCR conditions utilized 5% of the cDNA preparation in 10 mM Tris/HCl, pH 8.9, containing 50 mM KCl, 1.5 mM MgCl₂, 100 mg/ml gelatin, 0.2 mM dNTPs and the appropriate primers at 0.5 μM. A total of 35 cycles of the following parameters were used: 94 °C for 90 s; cool to 52 °C over 60 s; 52 °C for 90 s; rapidly heat to 72 °C and hold for 150 s; rapidly heat to 94 °C. The resultant PCR products were cloned into pUC18 and subjected to DNA sequence analysis.

Screening of cDNA libraries

Approximately 5 × 10⁴ rat epididymal and 2 × 10⁴ monkey testicular cDNA clones were transferred to nitrocellulose filters and screened for those hybridizing to the slightly larger of the pig NAD⁺-ICDH_γ PCR products, labelled with [³²P]dCTP, using a random priming protocol. Hybridization was for 12 h at 58 °C in 6 × SSC (1 × SSC is 0.15 M NaCl/0.015 M sodium citrate). After clone purification by subsequent secondary and tertiary screening under the same hybridization conditions, plasmid DNA from each strongly hybridizing clone was isolated and its cDNA sequence determined.

DNA sequence determination

All DNA sequencing was carried out on both DNA strands using a custom primer walking strategy and employing a Du Pont Genesis 2000 automated sequencer utilizing fluorescently labelled dideoxynucleotides. Sequence alignments were carried out using the LASERGENE suite of programs (DNASTAR, West Ealing, London, U.K.).

RESULTS AND DISCUSSION

Cloning and sequence analysis of cDNAs encoding the γ-subunits of pig, rat and monkey NAD⁺-ICDH

The availability of partial peptide sequence data for pig heart NAD⁺-ICDH enabled the design of 11 oligonucleotide primers for PCR amplification of cDNA derived from pig heart total RNA. However, the limited nature of the peptide data available meant that most of these PCR primers were short and/or highly redundant. This may explain why only three of the 11 primers (see Figure 2) led to the successful PCR amplification of a specific NAD⁺-ICDH cDNA fragment, despite considerable efforts at optimizing PCR parameters. The successful primers were based on the amino acid sequences labelled 1, 2 and 3 in Figure 2. Both PCR products were of approx. 600 bp in length and were cloned into pUC18. On sequence analysis they were found to contain an internal region that corresponded to the NAD⁺-ICDH_γ peptide LGDGSFLQCK (Huang and Colman, 1990). The identities of the PCR products were further corroborated by their similarity to the sequences of yeast NAD⁺-ICDH (see Figure 2).

Although the above PCR fragments were obtained from pig RNA, the availability of rat and monkey cDNA libraries of proven quality (Perry et al., 1992a,b) led us to screen these for the corresponding NAD⁺-ICDH_γ homologues. After screening approx. 5 × 10⁴ rat epididymal and 2 × 10⁴ monkey testicular cDNA clones, five strongly hybridizing rat clones (designated prE-ICDH_γ1–5) and one monkey clone (designated pmT-ICDH_γ1) were identified, purified and subjected to automated DNA sequence analysis of their cDNA inserts.

The resulting rat NAD⁺-ICDH_γ cDNA sequence (compiled from the large overlapping clones prE-ICDH_γ1 and prE-ICDH_γ3; Figure 1a) contains a poly(A) tail at its 3' end and an open reading frame extending from nucleotides 1 to 1164. The deduced amino acid sequence contains a region corresponding to the N-terminal peptide sequence of the mature γ-subunit of pig heart NAD⁺-ICDH (see Figure 2). The absence of a potential initiation codon upstream of the region corresponding to this peptide strongly suggests that the cDNA is truncated at its 5' end, within the presumptive mitochondrial import sequence. Nucleotide and deduced amino acid sequences of the monkey NAD⁺-ICDH_γ cDNA indicate that it too is truncated at the 5' end, immediately upstream of the region encoding the putative N-terminus of the mature mitochondrial subunit (Figure 2). Both rat and monkey NAD⁺-ICDH_γ cDNAs are thus full-length with respect to their respective mature polypeptides.

The considerable amino acid sequence conservation between mammalian NAD⁺-ICDH_γ subunits supports the notion that rat and monkey mitochondrial import peptides are cleaved at a point equivalent to that in the pig (Figure 2). Mitochondrial import peptides are commonly cleaved two or three residues to the C-terminal side of basic residues (Hartl and Neupert, 1990); such a configuration exists at the putative cleavage site in NAD⁺-ICDH_γ from the rat as well as both yeast NAD⁺-ICDH subunits.

Comparison of mammalian and yeast ICDH sequences

The pig, monkey and rat clones presented here show 89–97% identity in their deduced amino acid sequences. Moreover, the mammalian NAD⁺-ICDH_γ subunits are as closely related to both yeast subunits as the yeast subunits are to each other, with 40–45% deduced amino acid sequence identity in each case (Figure 2).

The contribution of mammalian NAD⁺-ICDH_γ to the catalytic and regulatory properties of the holoenzyme is unknown. Comparison of available peptide sequence data from all three subunits (Huang and Colman, 1990) implies that they are closely related, with a subset of identical peptides being reported in the β and γ subunits. In addition, the three subunits possess a Gly-Xaa-Gly-Xaa-Gly motif in their N-terminal peptides (residues 23–27 in the rat and monkey γ sequences; Figures 1a and 1b). This motif is present in both yeast NAD⁺-ICDH subunits, in *E. coli* NADP⁺-ICDH and in the isopropylmalate dehydrogenase family of enzymes (Imada et al., 1991). Mammalian NAD⁺-ICDH is stimulated by increases in mitochondrial Ca²⁺ but the yeast enzyme is not (B. J. Nichols and R. M. Denton, unpublished work). Ca²⁺ binds to NAD⁺-ICDH with a stoichiometry of one Ca²⁺ per α₂βγ unit (Rutter and Denton, 1989b). Since there is no obvious Ca²⁺-binding motif in the γ subunit sequences presented here, it is possible that a Ca²⁺-binding site will be found within one of the two subunits of NAD⁺-ICDH which have yet to be cloned, or that more than one subunit contributes Ca²⁺ coordinating residues. Both Mg²⁺-isocitrate and adenine nucleotides are required for NAD⁺-ICDH to bind to Ca²⁺ (Rutter and Denton, 1989b), indicating that the enzyme may not possess a conventional Ca²⁺-binding motif.

(a)

1
 GCG ATA GCT GCT GGC AGT GCT GCA AAG GCA ATA TTC AAG CCA GCT CTC CTC TGC CGT CCT TGG GAG GTT CTG GCT GCC CAT GAG GCC CCC CGA AGG AGC ATT TCC
 Ala Ile Ala Ala Gly Ser Ala Ala Lys Ala Ile Phe Lys Pro Ala Leu Leu Cys Arg Pro Trp Glu Val Leu Ala Ala His Glu Ala Pro Arg Arg Ser Ile Ser
 -34 -30 -20 -10 -1 +1

150
 TCA CAA CAA ACA ATT CCT CCG TCT GCT AAG TAT GGT GGG CGG CAT ACA GTG ACT ATG ATT CCA GGG GAT GGC ATC GGC CCG GAG CTC ATG TTG CAT GTT AAG TCG GTA
 Ser Gln Gln Thr Ile Pro Pro Ser Ala Lys Tyr Gly Gly Arg His Thr Val Thr Met Ile Pro Gly Asp Gly Ile Gly Pro Glu Leu Met Leu His Val Lys Ser Val

200
 TTC AGG CAT GCA TGT GTG CCA GTG GAC TTT GAA GAG GTG CAT GTA AGC TCC AAC GCT GAT GAG GAG GAC ATC CGC AAT GCC ATC ATG GCC ATC CGC CGG AAC CGT GTG
 Phe Arg His Ala Cys Val Pro Val Asp Phe Glu Glu Val His Val Ser Ser Asn Ala Asp Glu Glu Asp Ile Arg Asn Ala Ile Met Ala Ile Arg Arg Asn Arg Val

300
 GCT CTA AAG GGC AAC ATC GAA ACA AAT CAT GAC TTG CCA CCA TCC CAC AAA TCC CCG AAC AAC ATC CTT CGT ACC AGC CTA GAC CTC TAT GCC AAC GTC ATC CAC TGT
 Ala Leu Lys Gly Asn Ile Glu Thr Asn His Asp Leu Pro Pro Ser His Lys Ser Arg Asn Asn Ile Leu Arg Thr Ser Leu Asp Leu Tyr Ala Asn Val Ile His Cys

400
 AAG AGT CTG CCA GGA GTG GTG ACC CGG CAC AAG GAC ATA GAC ATC CTC ATT GTG CCG GAA AAC ACA GAA GGC GAG TAC AGC AGC CTG GAG CAT GAG AGT GTA GCA GGA
 Lys Ser Leu Pro Gly Val Val Thr Arg His Lys Asp Ile Asp Ile Leu Ile Val Arg Glu Asn Thr Glu Gly Glu Tyr Ser Ser Leu Glu His Glu Ser Val Ala Gly
 110 120 130 140

500
 GTG GTG GAG AGC TTG AAG ATT ATC ACC AAA GCC AAG TCC CTG CGC ATT GCT GAA TAT GCT TTC AAG CTG GCC CAG GAG AGT GGG CGT AAG AAA GTG ACG GCT GTG CAC
 Val Val Glu Ser Leu Lys Ile Ile Thr Lys Ala Lys Ser Leu Arg Ile Ala Glu Tyr Ala Phe Lys Leu Ala Glu Ser Gly Arg Lys Lys Val Thr Ala Val His
 150 160 170 180 190

600
 AAG GCC AAC ATC ATG AAA CTG GGT GAT GGA CTC TTC CTC CAG TGT TGC AGG GAA GTG GCA GCC CGC TAC CCT CAG ATC ACC TTT GAT AGC ATG ATT GTG GAC AAC ACA
 Lys Ala Asn Ile Met Lys Leu Gly Asp Gly Leu Phe Leu Gln Cys Cys Arg Glu Val Ala Ala Arg Tyr Pro Gln Ile Thr Phe Asp Ser Met Ile Val Asp Asn Thr
 200 210 220

700
 ACA ATG CAG CTG GTA TCC CGG CCT CAG CAG TTT GAT GTC ATG GTG ATG CCT AAT CTC TAT GGT AAC ATT GTC AAC AAC GTC TGT GCA GGG CTA GTT GGA GGC CCA GGC
 Thr Met Gln Leu Val Ser Arg Pro Gln Gln Phe Asp Val Met Val Met Pro Asn Leu Tyr Gly Asn Ile Val Asn Asn Val Cys Ala Gly Leu Val Gly Pro Gly
 230 240 250

800
 CTT GTG GCT GGG GCC AAC TAT GGC CAT GTG TAT GCA GTA TTC GAG ACA GCT ACA AGG AAC ACA GGC AAA AGT ATT GCC AAT AAG AAC ATT GCT AAC CCT ACT GCC ACA
 Leu Val Ala Gly Ala Asn Tyr Gly His Val Tyr Ala Val Phe Glu Thr Ala Thr Arg Asn Thr Gly Lys Ser Ile Ala Asn Lys Asn Ile Ala Asn Pro Thr Ala Thr
 260 270 280 290

900
 TTG CTA GCA AGT TGC ATG ATG CTA GAC CAC CTC AAG CTC CAT TCC TAT GCC ACC TCC ATC CGA AAA GCT GTC TTA GCA TCC ATG GAC AAT GAA AAT ATG CAT ACC CCA
 Leu Leu Ala Ser Cys Met Met Leu Asp His Leu Lys Leu His Ser Tyr Ala Thr Ser Ile Arg Lys Ala Val Leu Ala Ser Met Asp Asn Glu Asn Met His Thr Pro
 300 310 320

1000
 GAC ATT GGA GGC CAG GGC ACC ACA TCC CAA GCC ATC CAG GAC ATC ATT CGT CAC ATC CGC ATC ATT AAT GGA CCG GCT GTG GAG GCC TAG CTATCCCTGCAGTTTGCTCAGTT
 Asp Ile Gly Gly Gln Gly Thr Thr Ser Gln Ala Ile Gln Asp Ile Ile Arg His Ile Arg Ile Ile Asn Gly Arg Ala Val Glu Ala End
 330 340 350 354

1100
 TGCTGTAGGACTCCCTTCTCATTTTAGCTCCAGCTAGCTTTGGGGACAGGCTCCAGAATAAAGCCACTTGTTGCCAAAAAATAAAAAAAAAAAAAAAAAAAAAAAAAA

(b)

1
 C ATC TCT TCA CAA CAA ACA ATT CCT CCG TCG GCT AAG TAT GGC GGG CCG CAC ACA GTG ACC ATG ATC CCA GGG GAT GGC ATT GGG CCG GAG CTC ATG CTG CAT GTC
 Ile Ser Ser Gln Gln Thr Ile Pro Pro Ser Ala Lys Tyr Gly Gly Arg His Thr Val Thr Met Ile Pro Gly Asp Gly Ile Gly Pro Glu Leu Met Leu His Val
 1- +1 10 20 30

150
 AAG TCC GTC TTC AGG CAC GCA TGT GTA CCA GTG GAC TTT GAA GAG GTG CAC GTG AGT TCC AAC GCT GAT GAA GAG GAC ATT CGC AAT GCC ATC ATG GCC ATC CGC CGG
 Lys Ser Val Phe Arg His Ala Cys Val Pro Val Asp Phe Glu Glu Val His Val Ser Ser Asn Asn Asp Glu Glu Asp Ile Arg Asn Ala Ile Met Ala Ile Arg Arg
 40 50 60 70

200
 AAC CGT GTG GCC CTG AAG GGC AAC ATT GAA ACC AAC CAT AAC CTG CCA CCG TCA CAC AAA TCT CGA AAC AAC ATC CTT CGC ACC AGC CTG GAC CTC TAT GCC AAT GTC
 Asn Arg Val Ala Leu Lys Gly Asn Ile Glu Thr Asn His Asn Leu Pro Pro Ser His Lys Ser Arg Asn Asn Ile Leu Arg Thr Ser Leu Asp Leu Tyr Ala Asn Val
 80 90 100

300
 ATT CAC TGT AAG AGC CTG CCA GGT GTG ACC CCG CAC AAG GAC ATA GAC ATC CTC ATT GTC CGG GAG AAC ACA GAG GGC GAG TAT AGC AGC CTG GAG CAT GAG AGC
 Ile His Cys Lys Ser Leu Pro Gly Val Val Thr Arg His Lys Asp Ile Asp Ile Leu Ile Val Arg Glu Asn Thr Glu Gly Glu Tyr Ser Ser Leu Glu His Glu Ser
 110 120 130 140

400
 GTG GCG GGA GTG GTG GAG AGC CTG AAG ATC ATC ACC AAG GCC AAG TCC CTG CGC ATT GCC GAG TAT GCC TTC AAG CTG GCG CAG GAG AGC GGG CGC AAG AAA GTG ACG
 Val Ala Gly Val Val Glu Ser Leu Lys Ile Ile Thr Lys Ala Lys Ser Leu Arg Ile Ala Glu Tyr Ala Phe Lys Leu Ala Gln Glu Ser Gly Arg Lys Lys Val Thr
 150 160 170

500
 GCT GTA CAC AAG GCC AAC ATC ATG AAA CTG GGC GAT GGG CTT TTC CTC CAG TGC TGC AGG GAG GTG GCA GCC CGC TAC CCC CAG ATC ACC TTC GAG AAC ATG ATT GTG
 Ala Val His Lys Ala Asn Ile Met Lys Leu Gly Asp Gly Leu Phe Leu Gln Cys Cys Arg Glu Val Ala Ala Arg Tyr Pro Gln Ile Thr Phe Glu Asn Met Ile Val
 180 190 200 210

600
 GAC AAC ACC ACC ATG CAG CTG GTG TCC CGA CCC CAG CAG TTT GAT GTC ATG GTA ATG CCC AAT CTC TAT GGC AAC ATT GTC AAC AAC GTC TGC GCA GGA CTG GTT GGG
 Asp Asn Thr Thr Met Gln Leu Val Ser Arg Pro Gln Gln Phe Asp Val Met Val Met Pro Asn Leu Tyr Gly Asn Ile Val Asn Asn Val Cys Ala Gly Leu Val Gly
 220 230 240 250

700
 GGC CCA GGC CTT GTG GCT GGG GCC AAC TAT GGC CAT GTG TAT GCA GTG TTT GAA ACG GCT ACG AGG AAC ACC GGC AAG AGT ATC GCC AAT AAG AAC ATC GCC AAC CCC
 Gly Pro Gly Leu Val Ala Gly Ala Asn Tyr Gly His Val Tyr Ala Val Phe Glu Thr Ala Thr Arg Asn Thr Gly Lys Ser Ile Ala Asn Lys Asn Ile Ala Asn Pro
 260 270 280 290

800
 ACG GCC ACC TTG CTG GCC AGC TGC ATG ATG CTG GAC CAC CTC AAG CTG CAC TCC TAT GCC ACT TCC ATC CGT AAG GCT GTC CTG GCG TCC ATG GAC AAT GAG AAT ATG
 Thr Ala Thr Leu Leu Ala Ser Cys Met Met Leu Asp His Leu Lys Leu His Ser Tyr Ala Thr Ser Ile Arg Lys Ala Val Leu Ala Ser Met Asp Asn Glu Asn Met
 300 310 320

900
 CAC ACT CCA GAC ATC GGG GGC CAG GGC ACA CCA TCT GAA GCC ATC CAG GAC ATT ATC CGC CAC ATC CGC GTC ATC AAC GGC CCG GCC GTG GAG GCC TAG GCTGGCCCTGG
 His Thr Pro Asp Ile Gly Gly Gln Gly Thr Thr Ser Glu Ala Ile Gln Asp Ile Ile Arg His Ile Arg Val Ile Asn Gly Arg Ala Val Glu Ala End
 330 340 350 354

1000
 GACCTCCTTGATTCCCTTCCACCCAGCCAGCCAGCTGGTAGGCAGAGCCAGAAATAAG

Figure 1 Nucleotide and deduced amino acid sequence of NAD⁺-ICDH_γ from (a) rat epididymis and (b) monkey testis

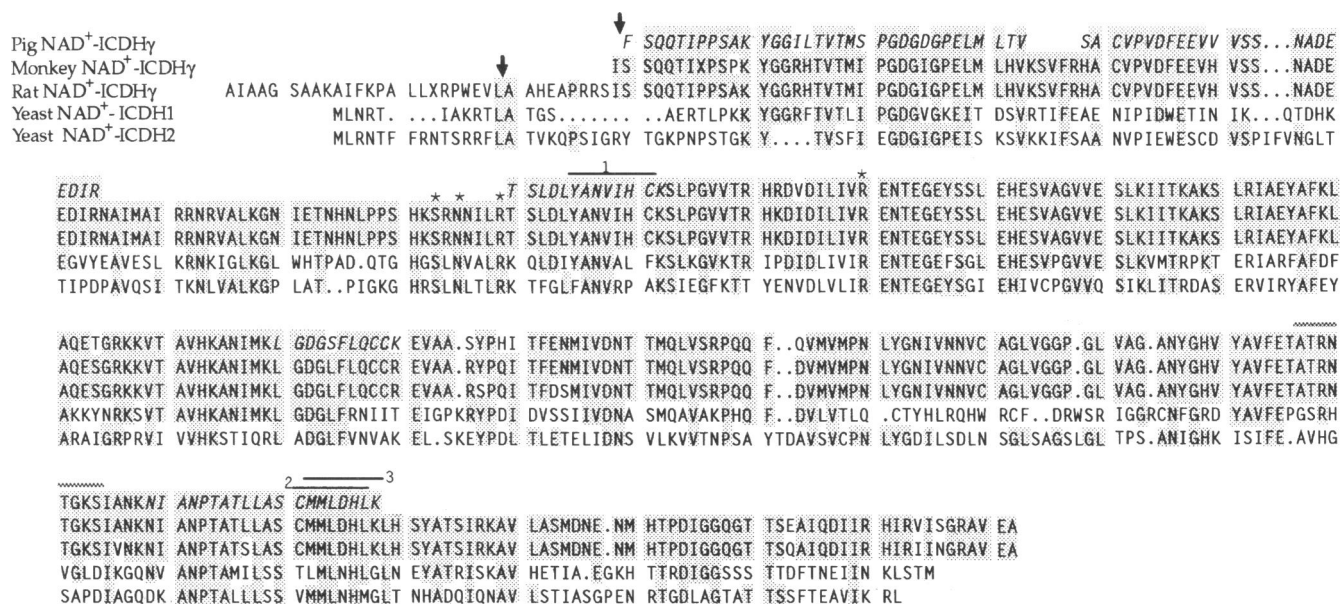


Figure 2 Comparison of amino acid sequences of NAD⁺-ICDH subunits from mammalian and yeast sources

The figure shows amino acid sequences from the γ subunit of the pig heart enzyme [from either direct peptide sequencing (italics; Huang and Colman, 1990) or deduced from a PCR product]; the γ subunit from monkey testis; the γ subunit from rat epididymis; and the two subunits of *S. cerevisiae* NAD⁺-ICDH. Shaded areas indicate sequence identity between mammalian and yeast subunits. Also indicated are the N-terminus of the pig heart and yeast subunits (arrows); the peptides from which the successful PCR primers used in this study were derived (numbered 1, 2 and 3); the residues involved in isocitrate binding, by analogy to *E. coli* NADP⁺-ICDH (*); and a possible ATP binding P-loop (bar; ATRNTGKS).

Mammalian NAD⁺-ICDH_γ possesses a P-loop-type ATP-binding motif, Ala/Gly-Xaa-Xaa-Xaa-Gly-Lys-Ser/Thr (Walker et al., 1982), which is not present in either subunit of the yeast enzyme (Figure 2). However, since mammalian and yeast NAD⁺-ICDH enzymes are inhibited by increases in the ATP/ADP ratio within the mitochondrion, the functional significance of this motif is unclear.

Rat NAD⁺-ICDH_γ is 28% identical to homodimeric *E. coli* NADP⁺-ICDH (Thorsness and Koshland, 1987). A crystal structure for the *E. coli* enzyme complexed with both Mg²⁺-isocitrate and NADP⁺ is available at 2.5 Å (0.25 nm) resolution (Hurley et al., 1991). With the exception of Arg-129, residues which bind via hydrogen bonds or salt bridges to isocitrate in the *E. coli* enzyme are conserved in rat and monkey NAD⁺-ICDH_γ subunits as well as in both subunits of yeast NAD⁺-ICDH. Residues Ser-91, Asn-93, Arg-97 and Arg-128 in the rat and monkey subunits correspond to residues Ser-113, Asn-115, Arg-119 and Arg-153 in *E. coli* NADP⁺-ICDH (Figure 2). In contrast, residues which co-ordinate to the Mg²⁺ of Mg²⁺-isocitrate are rather less well conserved.

Collectively, the deduced amino acid sequences for NAD⁺-ICDH_γ presented here provide evidence for the existence of both ATP- and isocitrate-binding sites within this subunit, and show that the subunit is highly conserved between different mammalian species. Moreover, this conservation extends, albeit to a lesser extent, to both yeast NAD⁺-ICDH subunits and suggests an ancestral relationship to prokaryotic NADP⁺-ICDH.

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