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Flavin reductase: Sequence of cDNA from bovine liver and tissue distribution

(methemoglobin reductase/oxidative damage)

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ABSTRACT **Flavin reductase catalyzes electron transfer from reduced pyridine nucleotides to methylene blue or riboflavin, and this catalysis is the basis of the therapeutic use of methylene blue or riboflavin in the treatment of methemoglobinemia. A cDNA for a mammalian flavin reductase has been isolated and sequenced. Degenerate oligonucleotides, with sequences based on amino acid sequences of peptides derived from bovine erythrocyte flavin reductase, were used as primers in PCR to selectively amplify a partial cDNA that encodes the bovine reductase. The template used in the PCR was first strand cDNA synthesized from bovine liver total RNA using oligo(dT) primers. A PCR product was used as a specific probe to screen a bovine liver cDNA library. The sequence determined from two overlapping clones contains an open reading frame of 621 nucleotides and encodes 206 amino acids. The amino acid sequence deduced from the bovine liver flavin reductase cDNA matches the amino acid sequences determined for erythrocyte reductase-derived peptides, and the predicted molecular mass of 22,001 Da for the liver reductase agrees well with the molecular mass of 21,994 Da determined for the erythrocyte reductase by electrospray mass spectrometry. The amino acid sequence at the N terminus of the reductase has homology to sequences of pyridine nucleotide-dependent enzymes, and the predicted secondary structure, $\beta\alpha\beta$, resembles the common nucleotide-binding structural motif. RNA blot analysis indicates a single 1-kilobase reductase transcript in human heart, kidney, liver, lung, pancreas, placenta, and skeletal muscle.**

Flavin reductase is a soluble enzyme that catalyzes electron transfer from pyridine nucleotides to flavins and a variety of other electron acceptors including methylene blue, 2,6-dichlorophenolindophenol (DCIP), and pyrroloquinoline quinone (PQQ). This catalysis is the basis of the therapeutic use of riboflavin and methylene blue in the treatment of congenital and acquired methemoglobinemia (see ref. 1 for review). For its role in the treatment of methemoglobinemia, the enzyme is also referred to as a methemoglobin reductase. However, under physiological conditions in the absence of a redox mediator, methemoglobin reduction is catalyzed not by flavin reductase but rather by a cytochrome b_5/cytochrome b_5 reductase system (1). There is clear evidence that the methylene blue-stimulated reduction of methemoglobin is dependent on the pentose phosphate pathway, indicating that the reductase is NADPH-dependent (2–4). The reductase has been isolated to varying degrees of purity from human (5–11), cattle (12–15), bullfrog (16), and blue white dolphin (17) erythrocytes and has been immunochemically detected in liver and heart tissues of cattle, rat, and rabbit (15, 18). Early reports on preparations of the enzyme showed higher rates of reduction of methylene blue with NADPH than with NADH (5, 6), but subsequent purifications indicated that the pyridine nucleotide specificity is dependent upon the electron acceptor and the species from which the reductase is isolated (7, 9, 10, 12, 13, 16).

Recently, it has been shown that administered riboflavin and PQQ protect heart, lung, and brain from cellular oxidative injury, and the protection has been proposed to be mediated through flavin reductase (18, 19). Higher oxidation states of hemeproteins have been implicated in the oxidative damage that occurs when a tissue is reoxygenated after an ischemic interval (20). It has been demonstrated that higher oxidation states of hemeproteins are rapidly reduced by dihydroriboflavin and $PQQH_2$, products of reductase-catalyzed reduction of riboflavin and PQQ (18, 21). Furthermore, hematoporphyrin, an inhibitor of the reductase, has been shown to prevent the protection by riboflavin in reperfused isolated rabbit hearts (22). These results suggest that riboflavin and PQQ may have great potential as therapeutic agents for the prevention of oxidative damage during cardiac arrest, myocardial infarction, stroke, acute lung injury, tissue inflammation, and organ transplantation and that flavin reductase plays a role in this prevention. Whereas the physiological role of flavin reductase has not been established, its observed activities suggest possible roles in protecting cells from oxidative damage or in regulating iron metabolism (22).

Although the activity of flavin reductase has been studied for >60 years, little is known about the primary structure of the reductase. Previously, we determined a partial amino acid sequence for bovine erythrocyte reductase (14). Here, we report the complete amino acid sequence of bovine liver reductase deduced from the nucleotide sequence of a cDNA clone for the reductase.† Based on amino acid sequence homology to other pyridine nucleotide-dependent enzymes, we identify a region of the reductase that binds the ADP moiety of the nucleotide cofactor. We also present data that indicate that the reductase is present in nonerythroid tissues.

MATERIALS AND METHODS

Determination of Molecular Mass by Electrospray Mass Spectrometry. Bovine erythrocyte flavin reductase (BLFR) was purified as described (14). Electrospray ionization mass spectra of the purified enzyme were obtained at the University of Michigan Protein and Carbohydrate Structure Facility using a Vestec electrospray source and a model 201 single quadrupole mass spectrometer (Vestec, Houston) fitted with a 2000 m/z range (23, 24).

Isolation and Sequence Determination of Peptides from Partial Acid Hydrolysis. Intact reductase, 1000 pmol, was

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Abbreviations: DCIP, 2,6-dichlorophenolindophenol; PQQ, pyrroloquinoline quinone; BLFR, bovine liver flavin reductase; GCG, Genetics Computing Group.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L35045).

hydrolyzed in 70% formic acid at 37°C for 24 hr (25). The resulting peptides were separated by 10–20% SDS/PAGE, electrotransferred to a poly(vinylidine difluoride) membrane (ProBlott/Immobilon P; Applied Biosystems), and stained with Coomassie blue. Peptide bands were excised from the membrane and subjected to automated Edman degradation on an Applied Biosystems model 473A instrument at the University of Michigan Protein and Carbohydrate Structure Facility.

Amplification by Polymerase Chain Reaction (PCR) of a Partial cDNA for the Reductase. Bovine liver total RNA was kindly provided by Kevin Walton (Department of Biological Chemistry, University of Michigan). First strand cDNA was synthesized using the cDNA cycle kit version 2.1 (Invitrogen) and oligo(dT) primers in a Perkin–Elmer/Cetus DNA thermal cycler 480. This first strand cDNA was used as the template in PCR to selectively amplify a partial cDNA for the reductase. The sense primer was based on residues 24–30 from the N-terminal region of the protein, QAGYEVT (14). This primer had a 512-fold degeneracy and included at its 5′ end a *Bam*HI restriction endonuclease site: 5′-CGCGGATCCCA(A/G)GC(N)GG(N)TA(T/C)GA(A/G)GT(N)AC-3′ (all oligonucleotides were synthesized at the University of Michigan Biomedical Research Core Facilities). The antisense primer was based on a 7-residue sequence, KYVAVMP, derived from a Lys-C endopeptidase-derived peptide (14). This primer had a 256-fold degeneracy and included at its 5′ end an *Eco*RI restriction endonuclease site: 5′-CCGGAATTCGGCAT(N)AC(N)GC(N)AC(A/G)TA(T/C)TT-3′. The PCR mixture contained AmpliTaq DNA polymerase (Perkin–Elmer) and 1.5 mM $MgCl_2$. The reaction was carried out for 30 cycles: denaturation step at 94°C for 1.5 min, annealing step at 52°C for 1.5 min, and elongation step at 72°C for 1 min. The PCR products were isolated by electroelution onto a DEAE membrane (Schleicher & Schuell) and cloned into a pBluescript II KS+ plasmid between *Eco*RI and *Bam*HI sites. The cloned PCR products were sequenced by the dideoxy chain-termination method (26) using the Sequenase 2.0 kit (United States Biochemical).

Screening a Bovine Liver cDNA Library. A bovine liver cDNA library, in λZAP II vector, was obtained from Stratagene and screened with a 381-bp PCR product. The PCR product was labeled with [α-^{32}P]dCTP (Amersham). The plaques were lifted in duplicate onto nitrocellulose filters (Schleicher & Schuell) and hybridized with the labeled probe at 42°C for 15 hr (27). Selected rescued phagemids (pBluescript) were isolated and the inserts were sequenced.

Calculation of the Mass of the Deduced Peptide. The theoretical mass of the bovine liver reductase polypeptide was calculated using the PROCOMP version 1.2 software program by Philip Andrews (University of Michigan Protein and Carbohydrate Structure Facility).‡

Data Base Searching and Sequence Analysis. Data base searching of the Protein Identification Resource, SwissProt, GenBank, and European Molecular Biology Laboratory was performed using the FASTA, TFASTA, and WORDSEARCH programs (28, 29) from the University of Wisconsin Genetics Computing Group (GCG) on the Clinical Research Computing VAX at the Medical School of the University of Michigan. Data base searching was also performed using the BLAST program from the National Institutes of Health (30). A Chou–Fasman prediction of secondary structures (31) was performed using software from the GCG program.

Northern Blot Analysis. A human multiple tissue Northern blot was obtained from Clontech. After prehybridization, the blot was hybridized at 42°C for 24 hr with a 381-bp PCR product that was labeled with ^{32}P. The blot was washed with 0.5× standard saline citrate containing 0.1% SDS at 65°C and exposed to film for 4 days.

‡Andrews, P. C., Third Meeting of the Protein Society, July 29–Aug. 2, 1989, Seattle, abstr. M139.

RESULTS

Determination of Molecular Mass of the Reductase Protein. Using electrospray mass spectrometry the molecular mass of the bovine erythrocyte reductase polypeptide was determined to be 21,994 ± 7 Da. The protein sample was run under conditions that do not maintain noncovalent complexes, so any cofactors, metal ions, or substrates associated with the protein would not be included in this mass determination.

Isolation and Sequence Determination of Two Peptides from the Reductase. From previous N-terminal amino acid sequencing of the erythrocyte protein (14), residues 35–36 were known to be Asp-Pro, which possess an acid-labile peptide bond. The protein was subjected to partial acid hydrolysis and the resulting peptides were separated by SDS/PAGE (data not shown). One of the resulting peptides, designated A1, ran as expected at a molecular mass of ≈3.8 kDa smaller than the intact reductase. Analysis of peptide A1 for 22 cycles revealed the sequence PSRLPSEGPQPAHVVVGDVRQP--. Analysis of another peptide, A2, revealed the sequence PSKVPPRLQD--.

Amplification of a Partial cDNA for Bovine Liver Reductase Using PCR. A partial cDNA was synthesized using PCR and degenerate oligonucleotide primers based on the amino acid sequences of peptides from the erythrocyte protein. The template for the PCR was first strand cDNA made using oligo(dT) primers and total RNA from bovine liver. A 381-bp PCR product was isolated and sequenced. One of the reading frames was identified as encoding a polypeptide with amino acid sequence identical to those of the reductase-derived peptides. It was later found that this PCR product corresponds to nucleotides 123–503 of the BLFR24 clone.

Isolation and Characterization of cDNAs Coding for Bovine Liver Reductase. A bovine liver cDNA library was screened with the PCR-generated partial cDNA encoding bovine flavin reductase cDNA. The initial screening of 1.4×10^6 plaques produced 34 positive clones. The nine clones with the strongest hybridization signals were subjected to secondary and tertiary screenings. Nucleotide sequences were obtained on four clones with the longest cDNA inserts; two clones, BLFR19 and BLFR24, were completely sequenced (Figs. 1 and 2). Clone BLFR24 is 834 nt long [excluding the poly(A) region] and contains the complete coding region. Clone BLFR19 is shorter than clone BLFR24, corresponding to nt

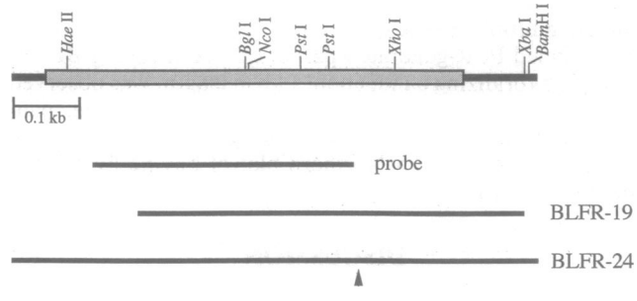

FIG. 1. Restriction map of cDNAs coding for BLFR. The 5′ and 3′ nontranslated regions are shown by the solid bar and the open reading frame is shown by the shaded bar. Probe is the PCR-generated partial cDNA used in screening the bovine liver cDNA library and in hybridizing to the human multiple tissue Northern blots. The probe corresponds to nt 123–503 of the cDNA. BLFR19 and BLFR24 are two independent clones isolated from the bovine liver cDNA library. BLFR19 corresponds to nt 190–756 of the cDNA. The arrowhead indicates the position in BLFR24 where additional sequence was found.

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CCCCCTCGTGTCCTGCGACTCCACGGCCTCGCGCCCCCACGATCCCGCAGC ATG GTC GTC AAG AAG ATT GCC CTT TTC GGC GCC ACC GGC   90
                                                    Met Val Val Lys Lys Ile Ala Leu Phe Gly Ala Thr Gly
                                                         1                                  10

AAC ACC GGG CTC ACC ACG CTG GCG CAG GCG GTG CAA GCA GGC TAT GAG GTG ACG GTG CTG GTG CGG GAC CCC TCC AGG CTG CCC TCA GAG  180
Asn Thr Gly Leu Thr Thr Leu Ala Gln Ala Val Gln Ala Gly Tyr Glu Val Thr Val Leu Val Arg Asp Pro Ser Arg Leu Pro Ser Glu
                         20                                     30                                     40

GGG CCC CAG CCG GCC CAC GTG GTG GTG GGT GAC GTC CGG CAG CCG GCC GAT GTG GAC AAG ACC GTG GCT GGG CAG GAC GCT GTC ATC GTG  270
Gly Pro Gln Pro Ala His Val Val Val Gly Asp Val Arg Gln Pro Ala Asp Val Asp Lys Thr Val Ala Gly Gln Asp Ala Val Ile Val
                         50                                     60                                     70

CTG CTG GGC ACC CGC AAT GAC CTC AGT CCT ACC ACA GTG ATG TCC GAG GGT GCC CAG AAC ATT GTG GCA GCC ATG AAG GCC CAT GGC GTG  360
Leu Leu Gly Thr Arg Asn Asp Leu Ser Pro Thr Thr Val Met Ser Glu Gly Ala Gln Asn Ile Val Ala Ala Met Lys Ala His Gly Val
                         80                                     90                                     100

GAC AAG GTC GTG GCC TGC ACC TCG GCC TTC CTA CTG TGG GAC CCT TCC AAG GTG CCC CCA AGA CTG CAG GAT GTG ACT GAT GAC CAT ATC  450
Asp Lys Val Val Ala Cys Thr Ser Ala Phe Leu Leu Trp Asp Pro Ser Lys Val Pro Pro Arg Leu Gln Asp Val Thr Asp Asp His Ile
                         110                                    120                                    130

CGG ATG CAC AAG GTA CTG CAG CAG TCG GGC CTG AAG TAC GTG GCC GTG ATG CCA CCA CAT ATA GGA GAC CAC CCA TTG ACT GGG GCC TAC  540
Arg Met His Lys Val Leu Gln Gln Ser Gly Leu Lys Tyr Val Ala Val Met Pro Pro His Ile Gly Asp His Pro Leu Thr Gly Ala Tyr
                         140                                    150                                    160

ACA GTG ACC CTG GAT GGA CGA GGG CCC TCA AGA GTC ATC TCC AAA CAT GAC CTG GGC CAC TTC ATG CTG CAC TGC CTC ACC ACC GAT AAG  630
Thr Val Thr Leu Asp Gly Arg Gly Pro Ser Arg Val Ile Ser Lys His Asp Leu Gly His Phe Met Leu His Cys Leu Thr Thr Asp Lys
                         170                                    180                                    190

TAC GAC GGG CAC ACC ACC TAC CCC TCC CAT GTG TAT GAA TAG GGCTCTGGCCCTGTCCCAGGAAGACAGCATCCTGTGAAACAGAACAAAGGAAGGGGGCAATAA  734
Tyr Asp Gly His Thr Thr Tyr Pro Ser His Val Tyr Glu  *
                         200             205

ACCTAGAGCCAAGAGCTTCAAATTACTCTAGAGGATCCACAGCT(A)n  779
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FIG. 2. cDNA and deduced amino acid sequence of BLFR. Nucleotides are numbered to the right of each line and amino acids are numbered below the corresponding residues. Nucleotides are numbered beginning with the first nucleotide in the cDNA clone BLFR24. Amino acids are numbered from the first Val, rather than the Met at the N terminus, since Val is the first amino acid observed in the isolated protein. The dotted underlined region has homology with the Kozak sequence (32). The stop codon is indicated by an asterisk. The underlined region marks the putative poly(A) signal sequence, which is located 48 nt residues 5′ of the poly(A) region found in BLFR24. The arrowhead indicates the position of additional sequence found in clone BLFR24. The double underlined amino acid sequence regions match the amino acid sequences determined by Edman degradation of the N-terminal region (14), Lys-C digest-derived peptides (ref. 14 and, for residues 137–144, unpublished results), and partial acid hydrolysis-derived peptides (this study).

190–756 of clone BLFR24. Clone BLFR24 contains an interruption of 65 nt in the open reading frame between nt 515 and 516, which are part of the codon for Gly-154. The junctions of this insertion are consistent with the conserved intron/exon splice junctions (33).

The first AUG could serve as the initiation codon for translation. The open reading frame contains 621 nt and encodes 206 residues. The peptide sequences of the erythrocyte reductase determined previously (14) and in this paper (double underlined in Fig. 2) match perfectly with the amino acid sequence of the liver reductase deduced from the cDNA. The mass of the deduced polypeptide, excluding the initial Met, was calculated to be 22,001 Da.

Detection of Flavin Reductase in Additional Tissues. A human multiple tissue poly(A)$^+$ blot was probed with a radiolabeled PCR-generated partial cDNA encoding BLFR. A single hybridizing band, about 1 kb in length, was observed in human heart, kidney, liver, lung, skeletal muscle, pancreas, and placenta (Fig. 3). A hybridizing band of about 1 kb was also observed in a Northern blot of bovine liver RNA (data not shown).

DISCUSSION

We report here the cDNA sequence and deduced amino acid sequence for a mammalian flavin reductase. Previously, the reductase had been considered to be only an erythrocyte enzyme. Our prior detection of the reductase in bovine liver (15) allowed us to use a bovine liver cDNA library to isolate a cDNA that encodes for the reductase, obviating the need to make a bovine reticulocyte cDNA library. The complete agreement of the amino acid sequences determined for bovine erythrocyte reductase-derived peptides with the deduced amino acid sequence of bovine liver reductase suggests that the reductase proteins from these two tissues are identical. The close agreement between the observed molecular mass of the erythrocyte reductase, 21,994 Da, and the theoretical mass of the liver reductase, 22,001 Da, also supports this conclusion.

The amino acid sequence of the N-terminal region of bovine flavin reductase is homologous to the amino acid sequences of pyridine nucleotide-dependent enzymes in that

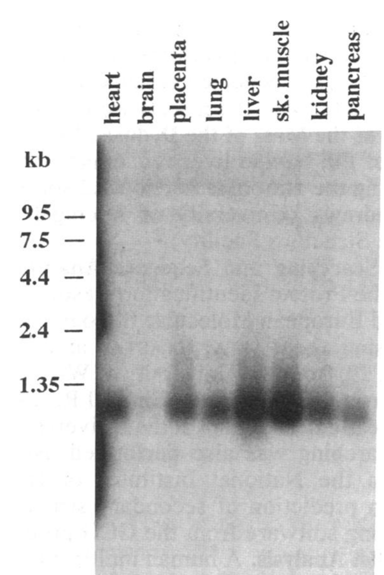

FIG. 3. Northern blot analysis of flavin reductase transcript in human tissue [poly(A)$^+$ RNA, 2 μg from each tissue indicated]. The blot was hybridized with a PCR-generated partial cDNA encoding bovine flavin reductase.

it has the characteristic arrangement of glycines and hydrophobic residues. The bovine flavin reductase sequence conforms to the 11 criteria for a nucleotide-binding sequence established by Wierenga *et al.* (34) with the exception of an additional residue between the first and second glycines in the fingerprint sequence (the first and second boxed G in Fig. 4). This "additional" residue is also present in malate dehydrogenase and UDPgalactose 4-epimerase, proteins whose tertiary structures are known and have the classic NAD-binding fold of a six-stranded parallel β-pleated sheet flanked on either side by α-helices (36, 39, 49). In these nucleotide-binding enzymes, the ADP moiety binds to a compact $\beta\alpha\beta$ fold that is held together by hydrophobic interactions; there are six nonpolar residues (the shaded residues in Fig. 4), two in each of the two β strands and two in the α-helix. Also, these enzymes have a sequence of GXGXXG/A or GXXGXXG/A, which forms the tight turn between the first β strand and the α-helix (the boxed glycines and alanines in Fig. 4). In addition to the general similarity of bovine flavin reductase to pyridine nucleotide-dependent enzymes, data base searches indicated homology to UDPgalactose 4-epimerase, dihydrodipicolinate reductase, isoflavone reductase, and dihydroflavonol-4-reductase. Further support for the proposal that the N-terminal region of bovine flavin reductase is a nucleotide-binding $\beta\alpha\beta$-fold is provided by the prediction of a $\beta\alpha\beta$ secondary structure for this region (Fig. 5).

Bovine flavin reductase shows homology to both NAD- and NADP-dependent enzymes. For many pyridine nucleotide-dependent enzymes, the nucleotide specificity can be predicted from the amino acid sequence, particularly from the nature of the last residue of the second β strand in the $\beta\alpha\beta$ fold (50–53). But the amino acid sequence of the putative second β strand of the flavin reductase can be aligned with both NAD- and NADP-dependent enzymes sequences (Fig. 4). An enzyme specific for NAD, such as UDPgalactose 4-epimerase, porcine cytosolic malate dehydrogenase, rat mitochondrial malate dehydrogenase, or glyceraldehyde-3-phosphate dehydrogenase, has as the last residue in the β strand an acidic amino acid residue whose carboxylate hydrogen bonds to the 2′ hydroxyl of the adenine ribose. An enzyme specific for NADP, such as dihydrodipicolinate reductase, isoflavone reductase, dihydroflavonol-4-reductase, or gluthatione reductase, has a noncharged amino acid at this position to avoid a charge repulsion that would occur between a carboxylate and the 2′ phosphate group of NADP. The homology of flavin reductase in this region to both NAD-dependent and NADP-dependent enzymes is consistent with the variability in specificity that has been reported for this enzyme. The nucleotide specificity of the reductase appears to vary with substrate and with species from which the enzyme is isolated.

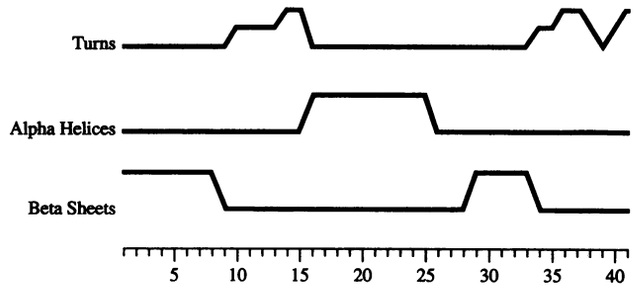

FIG. 5. Chou–Fasman prediction of secondary structure of the N-terminal region of bovine flavin reductase. The residue number corresponding to the secondary structure is given under the plot.

Aside from the identified pyridine nucleotide-binding sequence, the reductase did not show significant homology to any other proteins, including DT-diaphorases, PQQ-containing glucose dehydrogenases from bacteria, heme oxygenase, cytochrome b_5 reductase, cytochrome P-450 reductase, pyridine nucleotide transhydrogenases, and xanthine oxidase. Although bovine flavin reductase and a bacterial flavin reductase involved in ribonucleotide reduction (54) both catalyze flavin reduction, no sequence similarity was observed between these two proteins.

Protein	Sequence	Position	Nucleotide
FR (bovine)	VVKKIALFGATGNTGLTTLAQAVQAGYEV . . . . TVLVRDPSRLP	1-40	?
FR (bullfrog)	APKNIVLFGATGMTGQVTLGQALE	1-25	?
mMDH (rat)	AKVAVLGASGGIGQPLSLLLKNSPLVS . . . . RLTLYDIAHTP	1-38	NAD
* cMDH (pig)	EPIRVLVTGAAGQIAYSLLYSIGNGSVFGKDQPIILVLLDITPMM	2-46	NAD
* UDPgal4E (E.c.)	MRVVTGGSGYIGSHTCVQLLQNGH . . . . . DVIILDNLCNS	1-36	NAD
* LDH (dogfish)	SYNKITVVGV . GAVGMACAISILMKDLAD . . . . . EVALVDVMEDK	19-59	NAD
* GAPDH (lobster)	SKIGIDGF . GRIGRLVLRAALSCGAQ . . . . . VVAVNDPFIAL	1-36	NAD
secondary structure	β β β β β α α α α α α α α α α α α β β β β β β		
* GR (human)	LPGRSVIVGA . GYIAVEMAGILSALGS . . . . . KTSLMIRHDKV	186-222	NADP
DHDPR (E.c.)	ANIRVAIAGAGGRMGRQLIQAALALEGV . . . . . QLGAALEREGS	4-42	NADP
IFR (alfalfa)	TENKILILGPTGAIGRHIVWASIKAGN . . . . . PTYALVRKTPG	3-40	NADP
DFR (barley)	NKGPVVVTGASGFVGSWLVMKLLQAGY . . . . . TVRATVRDPAN	4-41	NADP
FR (bovine)	VVKKIALFGATGNTGLTTLAQAVQAGY . . . . . EVTVLVRDPSR	1-38	?

FIG. 4. Alignment of the amino acid sequence of flavin reductase to the amino acid sequences of other pyridine nucleotide-dependent enzymes. The shaded and boxed residues are 10 of the fingerprint positions designated by Wierenga *et al.* (34) for predicting that a sequence will form a nucleotide-binding $\beta\alpha\beta$ fold. Amino acids at the shaded positions are small and hydrophobic. The first and second boxes indicate invariant glycines. The third box indicates a conserved glycine or alanine. The boxed position at the end of the second β strand indicates the residue that confers some of the nucleotide specificity of the enzyme; a NAD-dependent enzyme has an acidic residue at this position, while a NADP-dependent enzyme has a nonpolar residue. An asterisk indicates that the tertiary structure of the protein is known by x-ray crystallography. The secondary structure is that of human glutathione reductase (35). Abbreviations used for secondary structure are as follows: α, α-helix; β, β strand. Abbreviations used for sequences are as follows: FR (bovine), flavin reductase from bovine (this paper); FR (bullfrog), partial sequence of flavin reductase from bullfrog (16); UDPgal4E (E.c.), UDPgalactose 4-epimerase from *Escherichia coli* (36, 37); cMDH (rat), cytosolic malate dehydrogenase from rat (38); mMDH (pig), mitochondrial malate dehydrogenase from pig (39); LDH (dogfish), lactate dehydrogenase from dogfish (40, 41); GAPDH (lobster), glyceraldehyde-3-phosphate dehydrogenase from lobster (42, 43); GR (human), glutathione reductase from human (44, 45); DHDPR (E.c.), dihydrodipicolinate reductase from *Escherichia coli* (46); IFR (alfalfa), isoflavone reductase from alfalfa (47); DFR (barley), dihydroflavonol-4-reductase from barley (48).

The Northern blot indicates the presence of the reductase in heart, kidney, liver, lung, pancreas, placenta, and skeletal muscle (Fig. 3). This observation confirms the previous immunochemical detection of flavin reductase in liver and heart (15, 18). The presence of the reductase in many tissues implies that the function of the enzyme is not erythrocyte-specific but more general.

Historically known for its role in the treatment of methemoglobinemia, the reductase may now be studied for its potential role in the treatment of oxidative tissue injury.

Note. A cDNA for human red cell flavin reductase has been cloned and sequenced (55).

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