The *udhA* Gene of *Escherichia coli* Encodes a Soluble Pyridine Nucleotide Transhydrogenase

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The *udhA* gene of *Escherichia coli* was cloned and expressed in *E. coli* and found to encode an enzyme with soluble pyridine nucleotide transhydrogenase activity. The N-terminal end of the enzyme contains the fingerprint motif of a dinucleotide binding domain, not present in published *E. coli* genome sequences due to a sequencing error. *E. coli* is hereby the first organism reported to possess both a soluble and a membrane-bound pyridine nucleotide transhydrogenase.

Pyridine nucleotide transhydrogenases catalyze the reversible transfer of reducing equivalents between NAD and NADP pools according to the following equation:

$$NADP^+ + NADH \rightleftharpoons NADPH + NAD^+ \qquad (1)$$

Based on the stereospecificity of the transfer, two groups of transhydrogenases have been defined. The AB transhydrogenases specifically transfer the 4A hydrogen of the nicotinamide ring of NADH to $NADP^+$ and the 4B hydrogen from NADPH to NAD^+, while the BB transhydrogenases transfer the 4B hydrogen from both NADPH and NADH (16). The AB transhydrogenases are found in mitochondria and in some bacteria, such as *Escherichia coli* (6). They are integral membrane proteins bound to the inner mitochondrial membrane or the bacterial membrane where they couple the transfer of hydride from NADH to $NADP^+$ with proton import (12). The genes encoding these enzymes have been cloned from numerous sources (1, 4, 27), facilitating the study of these membrane-bound transhydrogenases, and their physiological role is assumed to be the generation of NADPH, which can be used for reductive biosynthetic reactions.

The BB transhydrogenases are structurally unrelated to the AB transhydrogenases and have been found in *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Azotobacter vinelandii* (17). They are soluble flavoproteins containing flavin adenine dinucleotide (FAD) and are remarkable for their formation of large polymers. The enzyme from *P. aeruginosa* has a minimal active form of approximately 1.6×10^6 Da (23), probably composed of four stacked rings of seven or eight monomers (23), and upon isolation filaments exceeding 500 nm in length were observed (13). The *P. fluorescens* and *A. vinelandii* enzymes also form polymers (7, 21); however, the structure of the *A. vinelandii* polymers appears to be different from that of the *P. aeruginosa* enzyme (22). These soluble transhydrogenases (STH) also display interesting kinetic behavior, with NADPH and 2′-AMP strongly activating the enzyme and $NADP^+$ inhibiting its activity (24). Ca^{2+} is found to increase the activation and decrease the inhibition effect. The inhibition of STH by $NADP^+$ suggests that its physiological role is the

conversion of NADPH, generated by peripheral catabolic pathways, to NADH, which can enter the respiratory chain for energy generation (20).

The first soluble pyridine nucleotide transhydrogenase gene (*sth*) cloned was from *P. fluorescens* and revealed that the enzyme was related to the family of flavoprotein disulfide oxidoreductases (7). This family of enzymes includes the well-characterized dihydrolipoamide dehydrogenase, glutathione reductase, and mercuric reductase. These enzymes are active as homodimers and possess a characteristic redox-active disulfide bond. The subunits of these enzymes consist of an N-terminal FAD binding domain, a central NAD(P) binding domain, and a C-terminal dimerization domain (26). However, one of the cysteines involved in the redox-active disulfide bond characteristic of this family of enzymes was lacking in the *P. fluorescens sth* sequence. The *sth* sequence was found to be most similar to an *E. coli* gene of unknown function (*udhA*), showing 60% sequence identity and 77% similarity (7).

In order to investigate the identity of *udhA* from *E. coli*, and because no transhydrogenase activity could be detected in extracts of *E. coli* grown on rich medium under standard conditions, we cloned and overexpressed this gene in order to study its product.

Cloning and sequence analysis of *udhA* from *E. coli*. Based on the published *E. coli* genome sequence (GenBank no. U00006), oligonucleotides were designed in order to amplify by PCR the *udhA* gene from *E. coli* JM109 cells. The primers 5′-AGGGATCCAATAAAACGTCAGGGC-3′ and 5′-CCATCGATGGGGGTTGTTTATCTGC-3′ (with restriction sites underlined), annealing at positions approximately 150 bp upstream and downstream, respectively, of the potential structural gene, were used. PCR was performed with 30 s of denaturing (94°C), 30 s of annealing (55°C), and 90 s of polymerization (72°C) for 30 cycles, with an additional 90 s of denaturing prior to the first cycle (polymerase added after the 90 s) and 3 min of polymerization after the last cycle. The 1.6-kb PCR product was digested with *Bam*HI and *Cla*I and cloned into the multiple cloning site of pBluescript SK(+) (no. 52325; Stratagene). The resulting construct was designated pUDHA1 and the insert was sequenced in both orientations. The *udhA* sequence and the deduced amino acid sequence are given in Fig. 1. An open reading frame encoding a protein of 466 amino acids (including the initiating Met) was identified, with *E. coli*-like −35 and −10 promoter sequences and a Shine-Dalgarno sequence upstream of the initiating ATG (Fig. 1). The molecular mass of the protein excluding the initiating methionine was determined by the Genetics Computer Group PEPTIDESORT program

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     BamHI
     GGATCCAATA AAACGTCAGG GCAAAAGTAA GAAACAGACA AAGCAAAGGC  -104        GTGATCATGC ATCTGAAGTC GGGTAAAAAA CTGAAAGCTG ACTGCCTGCT  +797
                                                                        V  I  M   H  L  K  S   G  K  K   L  K  A   D  C  L  L

     CGCTCAGGAT ATAGCCAGAT AAATGACGGG GATCAATTGG CTTACCCGCG   -54        CTATGCCAAC GGTCGCACCG GTAATACCGA TTCGCTGGCG TTACAGAACA  +847
               -35                   -10        rbs                      Y  A  N   G  R  T   G  N  T  D   S  L  A   L  Q  N
     ATAAAATGTT ACCATTCTGT TGCTTTTATG TATAAGAACA GGTAAGCCCT   -4

     ACCATGCCAC ATTCCTACGA TTACGATGCC ATAGTAATAG GTTCCGGCCC   +47        TTGGGCTAGA AACTGACAGC CGCGGACAGC TGAAGGTCAA CAGCATGTAT  +897
        M  P   H  S  Y  D   Y  D  A   I  V  I   G  S  G  P              I  G  L   E  T  D  S   R  G  Q   L  K  V  N   S  M  Y

     CGGCGGCGAA GGCGCTGCAA TGGGCCTGGT TAAGCAAGGT GTGCGCGTCG   +97        CAGACCGCAC AGCCACACGT TTACGCGGTG GGCGACGTGA TTGGTTATCC  +947
        G  G   E  G  A  A   M  G  L   V  K  Q  G   V  R  V              Q  T  A   Q  P  H  V   Y  A  V   G  D  V   I  G  Y  P

     CAGTTATCGA GCGTTATCAA AATGTTGGCG GCGGTTGCAC CCACTGGGGC  +147        GAGCCTGGCG TCGGCGGCCT ATGACCAGGG GCGCATTGCC GCGCAGGCGC  +997
        A  V   I  E  R  Y   Q  N  V   G  G  G  C   T  H  W  G            S  L  A   S  A  A   Y  D  Q  G   R  I  A   A  Q  A

     ACCATCCCGT CGAAAGCTCT CCGTCACGCC GTCAGCCGCA TTATAGAATT  +197        TGGTAAAAGG CGAAGCCACC GCACATCTGA TTGAAGATAT CCCTACCGGT +1047
        T  I  P   S  K  A  L   R  H  A   V  S  R   I  I  E  F           L  V  K  G   E  A  T   A  H  L   I  E  D  I   P  T  G

     CAATCAAAAC CCACTTTACA GCGACCATTC CCGACTGCTC CGCTCTTCTT  +247        ATTTACACCA TCCCGGAAAT CAGCTCTGTG GGCAAAACCG AACAGCAGCT +1097
        N  Q  N   P  L  Y   S  D  H  S   R  L  L   R  S  S             I  Y  T   I  P  E  I   S  S  V   G  K  T   E  Q  Q  L

     TTGCCGATAT CCTTAACCAT GCCGATAACG TGATTAATCA ACAAACGCGC  +297        GACCGCAATG AAAGTGCCAT ATGAAGTGGG CCGCGCCCAG TTTAAACATC +1147
        F  A  D   I  L  N  H   A  D  N   V  I  N  Q   Q  T  R           T  A  M   K  V  P   Y  E  V  G   R  A  Q   F  K  H

     ATGCGTCAGG GATTTTACGA ACGTAATCAC TGTGAAATAT TGCAGGGAAA  +347        TGGCACGCGC ACAAATCGTC GGCATGAACG TGGGCACGCT GAAAATTTTG +1197
        M  R  Q   G  F  Y  E   R  N  H   C  E  I   L  Q  G  N           L  A  R  A   Q  I  V   G  M  N   V  G  T  L   K  I  L

     CGCTCGCTTT GTTGACGAGC ATACGTTGGC GCTGGATTGC CCGGACGGCA  +397        TTCCATCGGG AAACAAAAGA GATTCTGGGT ATTCACTGCT TTGGCGAGCG +1247
        A  R  F   V  D  E   H  T  L  A   L  D  C   P  D  G            F  H  R   E  T  K  E   I  L  G   I  H  C   F  G  E  R

     GCGTTGAAAC ACTAACCGCT GAAAAATTTG TTATTGCCTG CGGCTCTCGT  +447        CGCTGCCGAA ATTATTCATA TCGGTCAGGC GATTATGGAA CAGAAAGGTG +1297
        S  V  E  T   L  T  A   E  K  F   V  I  A  C   G  S  R           A  A  E   I  I  H   I  G  Q  A   I  M  E   Q  K  G

     CCATATCATC CAACAGATGT TGATTTCACC CATCCACGCA TTTACGACAG  +497        GCGGCAACAC TATTGGTAGC TTCGTCAACA CCACCTTTAA CTACCCGACG +1347
        P  Y  H   P  T  D  V   D  F  T   H  P  R   I  Y  D  S          G  G  N   T  I  E  Y   F  V  N   T  T  F  N   Y  P  T

     CGACTCAATT CTCAGCATGC ACCACGAACC GCGCCATGTA CTTATCTATG  +547        ATGGCGGAAG CCTATCGGGT AGCTGCGTTA AACGGTTTAA ACCGCCTGTT +1397
        D  S  I   L  S  M   H  H  E  P   R  H  V   L  I  Y            M  A  E   A  Y  R  V   A  A  L   N  G  L   N  R  L  F
                                                                                                                 termination sequence
     GTGCTGGAGT GATCGGCTGT GAATATGCGT CGATCTTCCG CGGTATGGAT  +597        TTAAAACTTT ATCGAAATGG CCATCCATTC TTGCGCGGAT GGCCTCTGCC +1447
        G  A  G  V   I  G  C   E  Y  A   S  I  F   R  G  M  D          *

     GTAAAAGTGG ATCTGATCAA CACCCGCGAT CGCCTGTTGG CATTTCTCGA  +647        AGCTGCTCAT AGCGGCTGCG CAGCGGTGAG CCAGGACGAT AAACCAGGCC +1497
        V  K  V   D  L  I  N   T  R  D   R  L  L   A  F  L  D

     TCAAGAGATG TCAGATTCTC TCTCCTATCA CTTCTGGAAC AGTGGCGTAG  +697        AATAGTGCGG CGTGGTTCCG GCTTAATGCA CGGCAGATAA ACAACCCCAT +1547
        Q  E  M   S  D  S   L  S  Y  H   F  W  N   S  G  V

     TGATTCGTCA CAACGAAGAG TACGAGAAGA TCGAAGGCTG TGACGATGGT  +747        CGAT +1551
        V  I  R  H   N  E  E   Y  E  K   I  E  G  C   D  D  G          ClaI
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FIG. 1. Sequence of *udhA* and deduced amino acid sequence of STH. Nucleotides are numbered, with 1 being the A of the initiating ATG. Restriction sites indicate the insertion sites in pBluescript SK(+). Putative promoter (−35 and −10) and terminator regions are indicated. *rbs*, ribosome binding site.

(8) to be 51,457 Da. The sequence differed from previously published genomic sequences (GenBank no. U00006, X66026, and AE000470) in having a 1-base deletion (a C) after nucleotide 40 (Fig. 1). This extra base in previously published sequences had given rise to a deduced protein sequence (P27306) which started at the methionine 22 amino acids downstream of the methionine presented in this study to be the start of the protein (see below). The N-terminal part of the protein missing in previously deduced sequences contains the amino acid sequence Gly-X-Gly-X-X-Gly/Ala, which is characteristic for a dinucleotide binding domain (Rossman fold). Homology with the disulfide pyridine nucleotide oxidoreductases (see Fig. 4) suggests that this is the FAD binding domain. The protein was found to be 59% identical and 77% similar to *P. fluorescens* STH.

Expression of recombinant STH in *E. coli*. Crude extracts were prepared from JM109/pUDHA1, grown to saturation in SOB medium (Difco) at 37°C, and assessed for STH activity. STH activity was assayed by monitoring the reduction of thionicotinamide adenine dinucleotide (tNAD$^+$) at 400 nm in a reaction mixture consisting of 0.1 mM NADPH and 0.1 mM tNAD$^+$ (Sigma Chemical Co.) in 50 mM Tris-HCl buffer (pH 7.0) at 30°C. The molar extinction coefficient of tNADH at 400 nm was taken as 11,300 liters mol^{-1} cm^{-1} (5). One unit of enzyme activity was defined as the amount of activity reducing 1 µmol of tNAD$^+$ per min under these conditions. Protein

concentration was measured by using a reagent from Pierce (Rockford, Ill.). Control extracts of JM109 grown under the same conditions did not have measurable STH activity (lowest detectable activity, approximately 0.001 U/mg) while extracts of the recombinant cells, JM109/pUDHA1, possessed about 2.9 U of STH activity per mg. This result implies that the *udhA* gene of *E. coli* encodes an enzyme with STH activity, and its putative product was therefore designated STH.

Purification and characterization of recombinant *E. coli* STH. The recombinant STH was purified to apparent homogeneity in a single affinity chromatography step by using adenosine-2′,5′-diphosphate agarose. The protocol used for the purification of *P. fluorescens* STH was applied except that the 0.7 M NaCl wash step was replaced by 0.5 M NaCl (7). A total of 1,600 U was loaded onto a column with a 6-ml packed volume in the presence of 5 mM CaCl$_2$. The most active eluted fractions, totalling 9.6 ml, were pooled and dialyzed against 50 mM Tris-HCl buffer (pH 7.0) with 5 mM dithiothreitol (DTT) in order to remove salts and reduce the pH. The product contained 1,150 U of STH at a specific activity of 85 U/mg. The specific activity of the purified *E. coli* STH is about 3.5-fold lower than that of the *P. fluorescens* STH under standard assay conditions (7). An expression level of about 3.5% of total soluble protein was estimated. The protein appeared to be homogeneous, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2), and the sub-
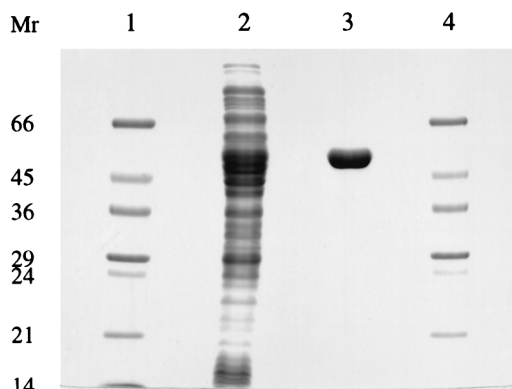
FIG. 2. SDS-PAGE showing purification of STH from *E. coli* JM109/
pUDHA1. Lanes 1 and 4, M_r markers; lane 2, crude extract (20 µg); lane 3,
purified STH (5 µg). Numbers on the left are in thousands.

unit M_r determined from SDS-PAGE correlates well with the
calculated size and with that reported for *P. fluorescens*, *P.
aeruginosa*, and *A. vinelandii* STHs (7, 17, 23). The N-terminal
sequence of the recombinant enzyme was determined by au-
tomated Edman degradation and was found to be P-H-S-Y-D-
Y-D-A-I-V-I-G-S-G-P-G-G-E-(R/G)-A-A-M-G-L-V-K. This
is consistent with the deduced amino acid sequence excluding
the initiating methionine and confirmed the translation start of
the *udhA* gene and the presence of a Rossman fold fingerprint
motif at the N terminus.

The UV-visible absorption spectrum of the purified STH
(Fig. 3) has a peak at 444 nm with a shoulder at 472 nm, and
it is characteristic of an oxidized flavoprotein (15). When the
enzyme was boiled and the denatured protein was removed by
centrifugation, the supernatant retained the yellow coloration
and showed the visible absorption spectrum of a free flavin.
The flavin liberated in this way was subjected to thin-layer
chromatography together with the flavin standards riboflavin,
flavin mononucleotide, and FAD. The flavin liberated from
STH comigrated with FAD and not with riboflavin or flavin
mononucleotide in two different thin-layer chromatography

systems (system I, 3% [wt/vol] $Na_2B_4O_7 \cdot 10H_2O$ in distilled
H_2O [dH_2O]; system II, *n*-butanol–dH_2O–acetic acid–metha-
nol [14:14:1:6]). This suggests that *E. coli* STH contains the
noncovalently bound prosthetic group FAD. This is also con-
sistent with earlier reports of STH from *P. aeruginosa* and *A.
vinelandii* (17). Assuming the extinction coefficient of FAD
given by Siegel (18), the absorbance spectrum suggests a flavin
content of 0.76 mol of FAD per mol of STH subunit.

In order to determine whether the *E. coli* STH was capable
of forming large polymers, as found for the *Pseudomonas* (7,
13) and *Azotobacter* (22) STHs, samples were adsorbed to
glow-discharged carbon Formar films from a 1.0-mg/ml solu-
tion, negatively stained with 1% (wt/vol) uranyl acetate, and
examined by transmission electron microscopy with a Philips
CM100 electron microscope operated at 80 kV. These electron
microscopy studies showed that the *E. coli* enzyme differs from
the other STHs in that it does not form filaments under these
conditions (data not shown).

In order to get an estimate of the native size of the *E. coli*
STH, gel filtration on Superose 6 10/30 (Pharmacia) was per-
formed. The column was equilibrated with 50 mM Tris-HCl
(pH 7.0) containing 100 mM NaCl and 5 mM DTT, and the
STH was dialyzed against the same buffer prior to applica-
tion. The column was calibrated with appropriate standards
(thyroglobulin, ferritin, catalase, aldolase, albumin, and
ovalbumin [from Pharmacia Biotech]; 200 µl of 1- to
5-mg/ml solutions), and STH (200 µl at a concentration of
1.0 mg/ml) was loaded, all at a flow rate of 0.2 ml/min. STH
was eluted as a broad peak with an average molecular mass
($\pm$ standard error) of 386 $\pm$ 31 kDa and a shoulder at the
monomer size, 51.6 $\pm$ 3.5 kDa (data not shown). This shows
that *E. coli* STH does form multimeric structures; however,
the enzyme is different from the *Pseudomonas* and *Azoto-
bacter* STHs as it is devoid of large polymers. The average
molecular weight suggests that the *E. coli* STH is present in
a form consisting of seven or eight monomers. As the *A.
vinelandii* STH is though to have a minimal active form
consisting of eight subunits (22), this could suggest that
these two STHs have a similar subunit arrangement.

Sequence comparisons. The deduced amino acid sequence
of the *E. coli* STH was compared to other sequences in protein
databases by using the BLAST 2 service of the National Center
for Biotechnology Information (NCBI) accessed at http://www
.bio.cam.ac.uk (Fig. 4). Various dihydrolipoamide dehydroge-
nases showed up to 27% identity and 45% similarity to the
E. coli STH. The purified *E. coli* STH did not show signif-
icant activity with lipoamide (<0.1 U/mg) in an assay system
consisting of 0.2 mM lipoamide and 0.2 mM NADH or
NADPH in 50 mM Tris-HCl (pH 7.0) at 30°C. Under the
same conditions, dihydrolipoamide dehydrogenase from
porcine heart (Sigma) displayed very high activity with
NADH (35 U/mg) but no significant activity with NADPH
(<0.3 U/mg). Dihydrolipoamide dehydrogenase did not dis-
play significant STH activity (<0.1 U/mg). Lack of dihydro-
lipoamide dehydrogenase activity is consistent with earlier
reports on STH (5, 7).

In contrast to the flavoprotein disulfide oxidoreductases (7),
both the *E. coli* and *P. fluorescens* STHs have a threonine at the
position of one of the redox-active cysteines characteristic of
this family (amino acids 50 and 49, respectively). The cysteine
residue missing in the STH sequences is directly involved in the
electron transfer between the nonnicotinamide substrate and
the FAD in dihydrolipoamide dehydrogenase, glutathione re-
ductase, and mercuric reductase. The conservation of the
threonine residue at this position in STH suggests that this
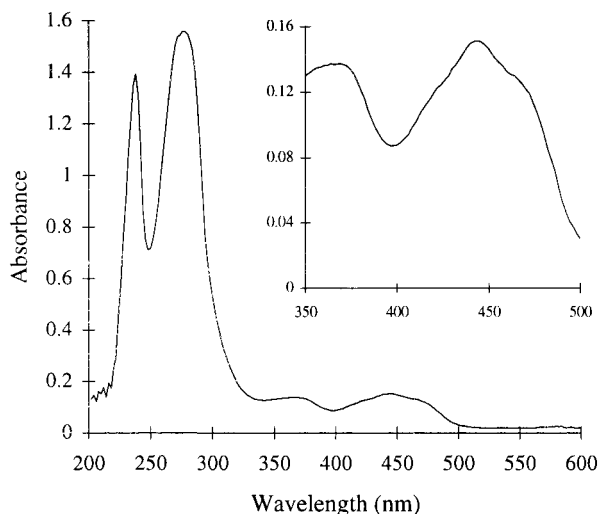residue might be of importance in the STH reaction. The

FIG. 3. UV-visible spectrum of STH. The spectrum of purified *E. coli* STH
(1.0 mg/ml) in 50 mM Tris-HCl buffer (pH 7.0), with 5 mM DTT and 10%
glycerol, was measured against a blank consisting of the same buffer. The inset
shows an enlargement of the region characteristic of an oxidized flavoprotein.

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mera_bacsr   1 MKKYRVNVQGMTCSGCEQHVAVALENMGAKAIEVDFRRGEAVFELPDDVKVEDAKNAIADANYHPGEAEEFQSEQKTNLLKKYRLNVEGMTCTGCEEHIA

                                                                                         ------
sth_ecoli    1 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GPHSYDYLAIVIGSGPGGEGAAMGLVKQGVRVAVIER
sth_psefl    1 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~MA.VYNYDVVVLGSGPAGEGAAMNAARAGRKVAMVDS
udha_myctu   1 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~MGSMREYDIVWIGSGPGGQKAAIASAKLGKSVAIVER
dldh_psefl   1 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~SQKFDVVVIGAGPGGYVAAIRAAQLGLKTACIEK
mera_bacsr 101 VALENAGAKGIEVDFRRGEALFELPYDVDIDIAKTAITDAQYQPGEAEEIQVQSEKRTDVSLNDEGNYDYIIIGSGGAAFSSAIDAVALNAKVAMIER
gshr_ecoli   1 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~MTKHYIAIGGSGCIASINRAAMYGQKCALIEA
nape_entfa   1 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~MKVIVLGSSHGGYEAVEELLNL.HPDAEIQW
                                                                             EEEEEEE  SHHHHHHHHHHHHHTT  EEEEE

                        *       *
sth_ecoli   38 YQ......NVGGGCTHWGTIPSKALRHAVSRIIEFNQN.PLYSDHSRLL.RSSFADILNHADNVINQCTR.MRQGFYBRNHCEILQCNARFVDEHILALD
sth_psefl   37 RR......QVGGNGTHLGTIPSKALRHSVRQIMQFNTN.EMFRAIGEPR.WFSFPDVLKEAEKVISKGVA.SRTGYYARNRVDLFFETGSEADEQIVEVV
udha_myctu  38 GR......MLGGVCVNTGTIPSKTLREAVLYLTGMNQR.ELYGASYRVKDRITPADLLARTQHVIGKEVD.VVRNQLMRNRVDLIVHGREIPPHEILVE
dldh_psefl  35 YIGKEGKVALGGTCLNVGCIPSKALLDSSYKYHEAKEA.FKVHGIEAKGVTIDVPAMVARKANIVKNLTG.GIATLFKANGVTSFEGHGKLLANKQVEVT
mera_bacsr 201 GT......VGGTCVNVGCVPSKTLLRAGEINHLAKNN.BFVGLHTSAS.NVDLAPLVKQKNDLVTEMRNEKYVNLIDDYGFEBIKCESKEVNENEVEVN
gshr_ecoli  36 KE......IGCTCVNVGCVPKKVMWHAAQIREAIHMYGDYGFDTTIN.KFNWETLIASRTAYIDRIHT.SYENVLGKNNVEVIKEFARFVEAKILEVN
nape_entfa  31 YE......KGDFISFLSCG..MQEYLEGKVKDVNSVR...YMTGEKME..S.RGVNVFRNTEITAIDPK.....EHQVTVKDIVSGEEFVENYDKLIIS
                  B SSSSB TTHHHHHHSHHHHHHHHHHHHHHHHHHHT SGGGTEEEEEEEE HHHHHHHHHHHHHHHHHHH HHHHHHHHHTEEEEEEEEEE GGGEEEEE

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sth_ecoli  129 CPDGS.VETLTAEKFVIACGSRFYHTTDVDFTHPRIYDSDSIILSMHHEBRHVLIYGAGVIGCEYASIFRGMDVKVDLINTRDRLL.AFLLQEMSDSLSYE
sth_psefl  128 CANGV.VEKPVAKHIIATGSRPYRPADIDFHHPRIYDSDTIISIGHTBRKLIIYGAGVIGCEYASIFSGLGVLVELVDNRIQLL.SFLDSBISQALSYE
udha_myctu 130 DQARREKTTVIGDYIIATGSRPARPSGVEFDEDRVLDSIGIILDLKSLPSSMVVVGAGVIGIEYASMBAALGTKVTVVEKRDNML.DFCLPEVVEALKFE
dldh_psefl 133 GLDGK.TQVITAENVIIASGSRPVEIPPAPLSDDILVDSTGALEFQAVBRKLIGKLGLGGVWARLGAKVTVLEALBKFL.PAADEQTAKEALKV
mera_bacsr 292 G......NQITAKRFLIATGASSTAENIPGLDEVDYLTSTSLLBLKKVPNRLTVIESGYIGMBLGQLBHNLCSBVTLIQRSERLL.KEYDPEISEAITKA
gshr_ecoli 127 G......BTIIPADHILIATGGRBSHEDIPGVEYG..IDSTGFFALPALFERVAVVGAGYIAVBLAGVINGLGAKTHLFVRKHAPL.RSFDPMISETDVEV
nape_entfa 111 P......GAVPFE...LDIPGKDLDNIYLMRGRQWAIKLKQKTVDPEVNN.VVWIGSCYIGIEAAEABAKACKKVIVIDILBRPLGVYLDKEFTDVLTEE
                ETTS   EEEEEEEEEE   EEE  BTTB   TTTEE HHHHTT SS  SEEEEE SHHHHHHHHHHHHHTT  EEEEE SSSSSS TTS HHHHHHHHHH

sth_ecoli  227 FWNSEVVIRHNEBYEKIEG.CDDGVIMHLK..SGK...KLKALCLIYANGRTGNTISLALQNIGLBTDSRGQLKVNSMYQTAQPHVYAVGDVIGYB....
sth_psefl  226 FSNNNITVRHNEBYDRVEG.LDNGVIHLDK..SGK...KIKALADLIWCNGRTGNTLKIGMENIEVKVNSRGQBEVDENYRIGVTNIYGAGDVIGWB....
udha_myctu 229 PRDLAVTFRFGEBVTAVDV.GSAGTVTTPA..SGK...QIPAETVMYSAGRQGQTBHLDHNAGLLVQGRGRHIEVDDRFQTKVDHIYAVGDVIGFP....
dldh_psefl 231 BTKQGLNIRLGARVTASFV.KKKQVTVTFTDANGEQKETF..IKLIVAVGRRPVTTDLLAADSGVTLDBRGFIYVDDHCKTSVPGVFAIGDVVRGA....
mera_bacsr 385 BTEQGINLVTGA..TYERVEQQGDIKKVHVEINGE.KRIIEABEOLIATGRKPIQTSLNLHAAGVEVGSRGEIVIDDYLKITNSRIYSAGDNTPGB....
gshr_ecoli 218 MNAEGPQLHTNAIPKAVVKNTDGSLTLELEDGR...SETV..ICLIWAIGREPANININLEAAGVKTNEKGYIVVDKYQNINIEGIYAVGDNTAV....
nape_entfa 201 MEANNITBIATGETVERYEG..GRVQKVVTDKN...A..YDABLVVVAVGVRPNTAWLKGT...LBLHPNGLIKTEYMRISEPDVFAVGDLATLIKYNPA
                HHHHT EEETT EEEE  E ETTEEEEEEE SS EEEEEE  S EEE S EEE  TTTB TTS    B TTS B B TT BBSSTTEEE GGGSSS

sth_ecoli  317 ......SLASAAYDQGRIAAQALVKG.EATAHLIEDIPTGIYTIPEISSVGKTEQQLTAM..KVPYEVGRAQBKHLARAQIVGMNVETLKILFHRETKEI
sth_psefl  316 ......SLASAAHDQGRSAAGSIVD..NGSWRYVNDVPTGIYTIPEISSIGKNEHBLTKA..KVPYEVCKAFFKSMARAQIAGEPQSMLKILFHRETLEV
udha_myctu 319 ......ALAATSMEQGRIAAYHAFG..EPTDGITELQEIGIYSIPEVSYVCATEVBLTKS..SIPYEVCVARYRELARGQIAGDSYGVFDPSGAFIQKL
dldh_psefl 324 ......MLAHKASEBCVMVAERIAG..HKAQMNYDLIBSVIYTHPEIAWVGKTEQTLKAE..GVENVCTFPBAASGRAMAANDTTGLVKVIADAKTDRV
mera_bacsr 478 ......QFVYVAAYBCGLAARNAIGG.LNQKVNLEVVBGVTFTSBSIATVGLTBQQAKEK..GYEVKTSVLPLDAVFRALVNRETTGVFKIVADAKTLKV
gshr_ecoli 309 ......BLTPVAVAACRRLSERLFNNKPDEHLDYSNIPTVVSHPBIGTVGLTBPQAREQYGDDQVKVYKSSFTAMYTAVTTHRQPCRMKIVCVGSEEKI
nape_entfa 291 DTEVNIALATNARKDGRFBVKNLEEP.VKPFPGVQGSSGLAVFDYKFASTGINBVMAQKL..GKETKAVTV.VEDYLMDFNPDKQKAWFKIVYDPBTTQI
                HHHHHHHHHHHHHHHTT     GGG  EEE SSSEEEEEE HHHHHHT  T  EEEEEEEGGG HHHHHHT     EEEEEEESSS BE

sth_ecoli  408 LGIHCFGER.AABIIHIGQAIMEQKFGGNTIEYFV.NTTFNYPTMAEAYRVAAINGLNRLF~~~~~~~~       Secondary structure
sth_psefl  406 LGVHCFGYQ.ASEIVHIGQAIMNQPGEONTLKYFV.NTTFNYPTMAEAYRVAAYDGLNRLF~~~~~~~~       elements:
udha_myctu 409 LGVHIFCTS.ATBMVHIGQAVM...HCBGSVEYLV.DAVFNYPTFSEAYKNAALDVMNKMRALNQFRR~
dldh_psefl 414 LGVHVICPS.AABLVCQGAIGM...EFGTSAFDLG.MMVBSHPTLSEALHBAALAVNGHAIHIANRKKR        H: α helix
mera_bacsr 569 LGAHVVAEN.AGDVIYAATLAV...KFGLIVGDLR.EBMAPYLTMAEGLKLAVLTFDKDVSKLSCCAG~       G: 3₁₀ helix
gshr_ecoli 403 VGIHGIGFG.MDBMLQGFAVAL...KMGATKKDFD.NTVAIHPTAABEFVTMR~~~~~~~~~~~~~~        E: H bonded ß strand
nape_entfa 387 LGAQLMSKADLTANINAISLAI...QAKMTIBDLAYADFBFQBAFDKPWNIINTAALEAVKQER~~~~~       B: isolated ß strand
                EEEEEEETT HHHHHHHHHHHHH   HHT BHHHHH TS   SS THHHHHHHHHHHHTT  TT                T: H bonded turn
                                                                                            S: bend
```

FIG. 4. Sequence alignment of *E. coli* STH with related enzymes. The alignment was generated by the CLUSTAL W program of the Genetics Computer Group package (8), and shading was applied by BOXSHADE; the black background shows identical residues and the grey background shows similar residues, both with a threshold of 0.5. The enzymes shown are as follows (with database accession numbers in parentheses): sth_ecoli, STH from *E. coli* (this study); sth_psefl, STH from *P. fluorescens* (GenBank U91523); udha_myctu, unknown dehydrogenase from *M. tuberculosis* (GenBank MTCY05A6; gene name, Rv2713); dldh_psefl, dihydrolipoamide dehydrogenase from *P. fluorescens* (Swissprot P14128); mera_bacsr, mercuric reductase from *Bacillus* sp. strain RC607 (Swissprot P16171); gshr_ecoli, glutathione reductase from *E. coli* (Swissprot P06715); nape_entfa, NADH peroxidase from *Enterococcus faecalis* (Swissport P37062). *, redox-active cysteine residues; -, Rossman fold Gly-X-Gly-X-X-Gly/Ala motifs forming the FAD and NAD(P) binding sites (14, 25). The bottom line shows the secondary structure elements aligned with dldh_psefl (from 1lpf.pdb).

STH sequences were found to be most similar to an unknown dehydrogenase sequence of *Mycobacterium tuberculosis*, showing 41% identity and 61% similarity to the *E. coli* STH. Interestingly, the *M. tuberculosis* sequence also possesses a threonine at the same position as the STHs.

To the best of our knowledge, the finding of an STH in *E. coli* makes this organism the first one reported to possess both a soluble and a membrane-bound transhydrogenase. The existence of two types of transhydrogenases in *E. coli* raises interesting questions about the relative functions of these two enzymes. It is very interesting that the 3′ end of *udhA* has a 12-nucleotide overlap with the 3′ end of the *oxyR* gene (10). Overlapping genes often have important regulatory implications, and this unusual overlap suggests that the two genes are possibly not expressed under the same conditions. The *oxyR* gene encodes the transcription factor OxyR, which regulates the expression of several antioxidant genes (e.g., those for catalase hydroperoxidase I and glutathione reductase) in re-

sponse to hydrogen peroxide (2, 3). The expression of *oxyR* is maximal in the exponential phase of aerobic growth, when cells are most likely to encounter oxidative stress (9). It has been demonstrated that it is the OxyR protein itself which is sensitive to hydrogen peroxide, activating the protein through the reversible formation of a disulfide bond (3, 28). The oxidized OxyR binds to promoter elements of the genes it regulates, resulting in the onset of their expression, and the protein also represses its own synthesis (3, 19). Hoek and Rydström proposed that the mitochondrial transhydrogenase is particularly important during oxidative stress (11), and we can in this respect speculate whether the STH and the membrane-bound transhydrogenase of *E. coli* have complementing functions in the cell. A study of the wild-type expression of *udhA* in *E. coli* under various conditions will assist in understanding the physiological role of this enzyme.

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