Volume 323, number 3, 289–293 FEBS 12536 June 1993

Evolutionary relationships between yeast and bacterial homoserine dehydrogenases

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Received 7 April 1993

The *Saccharomyces cerevisiae HOM6* gene, encoding homoserine dehydrogenase (EC 1 1.1 3) was cloned and its nucleotide sequence determined. The yeast homoserine dehydrogenase shows extensive homology to the homoserine dehydrogenase domains of the two aspartokinase-homoserine dehydrogenases from *Escherichia coli* as well as to the homoserine dehydrogenases from Gram positive bacteria. Sequence alignment reveals that the yeast enzyme is the smallest homoserine dehydrogenase known, owing to the absence of a C-terminal domain endowed with the L-threonine allosteric response in Gram positive bacteria. Accordingly, the *S. cerevisiae* enzyme appears to be a naturally occurring feedback resistant homoserine dehydrogenase. Our results indicate that homoserine dehydrogenase was originally an unregulated enzyme and that feedback control acquisition occured twice during evolution after the divergence between Gram positive and Gram negative bacteria.

Homoserine dehydrogenase; Evolution of enzyme function

1. INTRODUCTION

In *Saccharomyces cerevisiae*, as well as in bacteria, threonine, isoleucine and methionine derive some of their carbon atoms from aspartate. Reactions permitting the transformation of aspartate into homoserine thus constitute a common pathway to the biosyntheses of these amino acids. The last commited step of this common pathway appears to be the central point of regulatory mechanisms since most bacterial homoserine dehydrogenases exhibit end-product allosteric inhibition by threonine. This regulation seems to be achieved differently in Gram positive and Gram negative bacteria. The study of deregulated mutants of the *Corynebacterium glutamicum* enzyme shows that allosteric inhibition is mediated by a C-terminal domain [1,2]. A homologous domain is found on the homoserine dehydrogenase from *Bacillus subtillis* [3]. In contrast, in *E. coli*, there are two homoserine dehydrogenases which are both associated with aspartokinase in bifunctional enzymes [4,5]. These enzymes have been studied in detail and exhibit similar triglobular domain structures: an N-terminal domain carries the aspartokinase activity, a C-terminal domain is endowed with the homoserine dehydrogenase activity and a central inactive domain separates the two [6]. Allosteric inhibition of the *thrA*-encoded homoserine dehydrogenase seems to be mediated through the aspartokinase domain [7]. The homoserine dehydrogenase II from *E. coli*, encoded by the *metL* gene, is inhibited neither by threonine nor by methionine. However, homoserine dehydrogenase II synthesis is repressible by methionine [8].

Although homoserine dehydrogenase was early detected in *S. cerevisiae* [9], structural information has not been available until the recent study of Yumoto et al. [10]. The purified product appears to be composed of two identical subunits with a molecular weight of 40,000. The size of the yeast homoserine dehydrogenase thus appears to be comparable to the size of homoserine deshydrogenase from Gram positive bacteria rather than to that from *E. coli*. Therefore it seemed of interest to obtain molecular information about the yeast enzyme to understand the relationship existing between microbiological homoserine dehydrogenases.

We report here the cloning and sequencing of the *HOM6* gene, encoding the homoserine dehydrogenase from the yeast *Saccharomyces cerevisiae*. Comparison of its deduced amino acid sequence with those from bacteria reveals that the yeast enzyme is the smallest homoserine dehydrogenase known to date.

2. MATERIALS AND METHODS

2 1. *Strains, media and microbiological techniques*

E. coli strains HB101 and JM103 were used as hosts for plasmid maintenance, *S cerevisiae* strains used in this work are strain CC584-6B (*MATα, leu2, ura3, ade2, hom6*) and strain W303-1A (*MATa, his3, leu2, ura3, ade2, trp1*). To grow *S. cerevisiae*, YPG and YNB media were as described in Sherman et al. [11]. *S. cerevisiae* was transformed after lithium chloride treatment as described by Ito et al. [12]. Genetic crosses, sporulation, dissection and scoring of nutritional markers were as described by Sherman et al. [11].

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2.2 *Plasmids and phage vectors*

Plasmids pEMBLYi22, pEMBLYe23 [13] and pRS316 [14] were used as shuttle vectors between *S. cerevisiae* and *E. coli*. Bacteriophage M13mp18 and M13mp19 were used for single-stranded DNA production. The *S. cerevisiae* genomic library used for the cloning of the *HOM6* gene was constructed by inserting the product of a partial *Hin*dIII digest of chromosomal DNA from strain X2180-1A in the *Hin*dIII site of plasmid pEMBLYe23.

2.3. *Recombinant DNA methods*

Plasmid purification was performed as described by Ish-Horowicz and Burke [15]. To determine the sequence of *HOM6*, the *Xho*I–*Eco*RI fragment of pHOM6-5 was subcloned in bacteriophages M13mp18 and M13mp19. Systematic deletion subclones were generated as described in Thomas and Surdin-Kerjan [16] Single-stranded phage DNA prepared from these deleted clones was sequenced using the Pharmacia T7 sequencing kit. Analysis of the DNA sequence and comparisons on a VAX computer was made possible by the computer facilities of CITI2 in Paris [17] The nucleotide sequence of the *S cerevisiae HOM6* gene shown in Fig. 2 has been deposited with EMBL Bank under accession number X64457.

3. RESULTS

3.1. *Cloning and sequencing of the* HOM6 *gene*

The *HOM6* gene was cloned by complementation of the homoserine auxotrophy of the strain CC584-6B. This strain was transformed with the pEMBLYe23 based library described in section 2. Among 17,000 Ura^+ transformants tested, four strains were able to grow in the absence of homoserine. Three clones harbored identical plasmids with an insert of 5.7 kilobase pairs whereas one strain harbored a plasmid bearing the same genomic fragment but inserted in the reverse orientation. The insert was subcloned and the sequences required to complement the *hom6* mutation of strain CC584-6B were mapped to the *Xho*I–*Eco*RI fragment (Fig. 1). To demonstrate that the cloned fragment contains the *HOM6* gene, we determined whether this DNA sequence could integrate along with the plasmid vector at the *HOM6* locus by homologous recombination. The *Xho*I–*Hin*dIII fragment of pHOM6-1 was subcloned into the integrative vector pEMBLYi22 yielding plasmid pHOM6-4 (pEMBLYi22 only carries the *URA3* gene along with the sequence of plasmid pEMBL9. Therefore stable transformants result from integration of this plasmid into the genome). Strain W303-1A (*MATa, ura3, HOM6*) was transformed with the plasmid pHOM6-4 linearized by *Cla*I to direct integration to the homologous genomic sequences. A resulting Ura^+ transformant was crossed to strain CC584-6B (*MATα, ura3, hom6*). The diploid was sporulated and its meiotic progeny was analysed. No *hom6, URA3* recombinants were found in 23 tetrads analysed, confirming genetic linkage between the mutant locus and the cloned DNA.

The 2.8 kb *Xho*I–*Eco*RI fragment of pHOM6-1 was sequenced as described in section 2. This sequence was entirely determined on both strands and an open reading frame extending 1,070 bp was found (Fig. 2). The *HOM6* open reading frame is capable of encoding a protein composed of 359 amino acids with a predicted molecular mass of 38 kDa. In addition, the 3′ end (1,011 bp, 337 amino acids) of an another open reading frame was found 130 bp downstream the *HOM6* gene, the deduced polypeptide of which showed no significant homology with any protein in the NBRF protein data bank (release 34) using comparison program FASTP [18]. The predicted molecular weight of the *HOM6*-encoded product is thus identical to that of the purified subunit of the yeast homoserine dehydrogenase. Furthermore, the product predicted by the sequence of *HOM6* contains the two peptides identified by Yumoto et al. on the purified yeast homoserine dehydrogenase (Fig. 3).

3.2. *Amino acid sequence comparisons*

The sequence of homoserine dehydrogenase from *S. cerevisiae* was compared against the NBRF sequence collection (release 34). The sequences were aligned using the Macaw program [19]. Local alignments were opti-

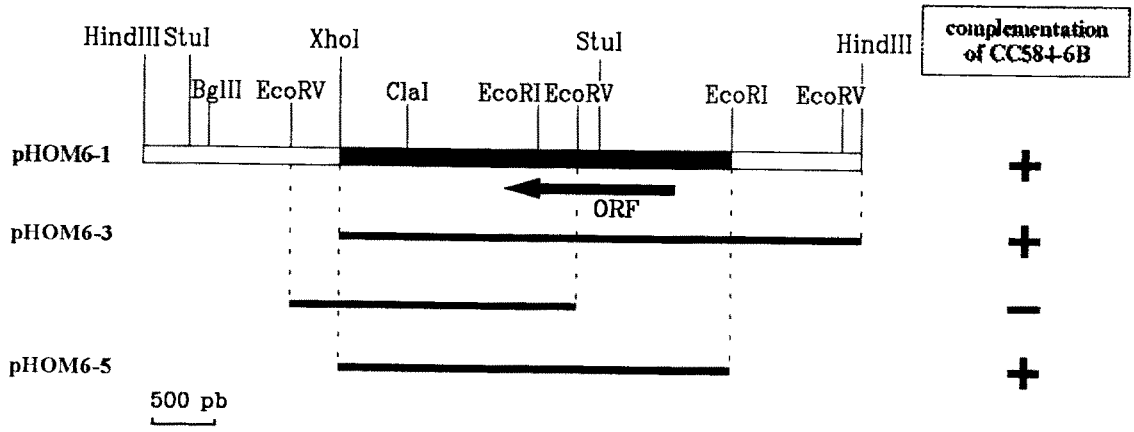

Fig. 1. Physical map of the *HOM6* region The fragments subcloned in pEMBLYe23 or pRS316 plasmids, as well as their ability to complement the *hom6* mutation of strain CC584-6B are shown.

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   ctgaagattagtgagcaactgaaccaagttttcgaccaaggaaagtaccctgaagaaatatcatccggg -505
ttaaaacttgctttccagttgaaaaagggacatagcggaattgcatttgacagcgtttgcttgggcatttat -433
ttcgagtatctctattttcctctagctcgacaggatcaatccctcacagacgtgaacgatgaaaacaatcca -361
gcactaccgtcatcaggttctgtgacatcaaaaagcactccagaccctacttctaagccaagtgcaattaag -289
aaacgtgttacaaagaaggaagtcttcgatagagtgaggctattagtggataagatcacgtgagtttttcaa -217
taacgcacatggtggtgtacgggtgtgttgatgaccatattttggaagaagtgtgccctggtggtaaagttg -145
ggttgaatggaacagaaatgtataaagaatatgtaaatattgcggttagcgatagacaatttgttgaagcta  -73
aatcttctgtgtgtgtactcaggactgttttaagaacgtagatagtatcatcaatcgaataataaaaaaaaa   -1
ATG AGC ACT AAA GTT GTT AAT GTT GCC GTT ATC GGT GCC GGT GTT GTT GGT TCA    54
 M   S   T   K   V   V   N   V   A   V   I   G   A   G   V   V   G   S
GCT TTC TTG GAT CAA TTG TTA GCC ATG AAG TCT ACC ATT ACT TAC AAT CTA GTT   108
 A   F   L   D   Q   L   L   A   M   K   S   T   I   T   Y   N   L   V
CTT TTG GCT GAA GCT GAG CGT TCT TTA ATC TCC AAG GAC TTT TCT CCA TTA AAT   162
 L   L   A   E   A   E   R   S   L   I   S   K   D   F   S   P   L   N
GTT GGT TCT GAT TGG AAG GCT GCT TTA GCA GCC TCC ACT ACT AAA ACG TTG CCT   216
 V   G   S   D   W   K   A   A   L   A   A   S   T   T   K   T   L   P
TTG GAT GAT TTA ATT GCT CAT TTG AAG ACT TCA CCT AAG CCA GTC ATT TTG GTT   270
 L   D   D   L   I   A   H   L   K   T   S   P   K   P   V   I   L   V
GAT AAC ACT TCC AGC GCT TAC ATT GCT GGT TTT TAC ACT AAG TTT GTC GAA AAT   324
 D   N   T   S   S   A   Y   I   A   G   F   Y   T   K   F   V   E   N
GGT ATT TCC ATT GCT ACT CCA AAC AAG AAG GCC TTT TCC TCT GAT TTG GCT ACC   378
 G   I   S   I   A   T   P   N   K   K   A   F   S   S   D   L   A   T
TGG AAG GCT CTT TTC TCA AAT AAG CCA ACT AAC GGT TTT GTC TAT CAT GAA GCT   432
 W   K   A   L   F   S   N   K   P   T   N   G   F   V   Y   H   E   A
ACC GTC GGT GCT GGT TTG CCT ATC ATC AGT TTC TTA AGA GAA ATT ATT CAA ACC   486
 T   V   G   A   G   L   P   I   I   S   F   L   R   E   I   I   Q   T
GGT GAC GAA GTT GAA AAA ATT GAA GGT ATC TTC TCT GGT ACT CTA TCT TAT ATT   540
 G   D   E   V   E   K   I   E   G   I   F   S   G   T   L   S   Y   I
TTC AAC GAG TTC TCC ACT AGT CAA GCT AAC GAC GTC AAA TTC TCT GAT GTT GTC   594
 F   N   E   F   S   T   S   Q   A   N   D   V   K   F   S   D   V   V
AAA GTT GCT AAA AAA TTG GGT TAT ACT GAA CCA GAT CCA AGA GAT GAT TTG AAT   648
 K   V   A   K   K   L   G   Y   T   E   P   D   P   R   D   D   L   N
GGG TTG GAT GTT GCT AGA AAG GTT ACC ATT GTT GGT AGG ATA TCT GGT GTG GAA   702
 G   L   D   V   A   R   K   V   T   I   V   G   R   I   S   G   V   E
GTT GAA TCT CCA ACT TCC TTC CCT GTC CAG TCT TTG ATT CCA AAA CCA TTG GAA   756
 V   E   S   P   T   S   F   P   V   Q   S   L   I   P   K   P   L   E
TCT GTC AAG TCT GCT GAT GAA TTC TTG GAA AAA TTA TCT GAT TAC GAT AAA GAT   810
 S   V   K   S   A   D   E   F   L   E   K   L   S   D   Y   D   K   D
TTG ACT CAA TTG AAG AAG GAA GCT GCC ACT GAA AAT AAG GTA TTG AGA TTC ATT   864
 L   T   Q   L   K   K   E   A   A   T   E   N   K   V   L   R   F   I
GGT AAA GTC GAT GTT GCC ACC AAA TCT GTG TCT GTA GGA ATT GAA AAG TAC GAT   918
 G   K   V   D   V   A   T   K   S   V   S   V   G   I   E   K   Y   D
TAC TCA CAC CCA TTC GCA TCA TTG AAG GGA TCA GAT AAC GTT ATT TCC ATC AAG   972
 Y   S   H   P   F   A   S   L   K   G   S   D   N   V   I   S   I   K
ACT AAG CGT TAC ACC AAT CCT GTT GTC ATT CAA GGT GCC GGT GCC GGT GCT GCC  1026
 T   K   R   Y   T   N   P   V   V   I   Q   G   A   G   A   G   A   A
GTT ACT GCC GCT GGT GTT TTG GGT GAT GTT ATC AAG ATT GCT CAA AGA CTT TAG  1080
 V   T   A   A   G   V   L   G   D   V   I   K   I   A   Q   R   L   *
atcagtaaacagacatataaaaacataggtatatttatatataaatagataacattatgctttactatccac 1152
gttcgaatattatttactttgtgaagaaactcatccatgtcatggggctactcaatcttctacaatctttcc 1224
tgtaatccacatttcctttgcgtcaaggaaaattgaccttaacttctgatagtccaagcaagttgatttgaa 1296
atttaggatcacttttgagcatctaatgaagagcttgaagcagaatgaattttcccgattatgtttcccgg 1368
attcactctggatatataaatgttgttaggagctaaaaataggcagccattttccctcaattccgcgacgta 1440
agcacccgtatgatgaatatattgaacatatttgaactgtggtttgccccatgaatacatgatgtcaaactc 1512
tatatcttgattttgtaaaaatttggatgccctattgtctaataaaaacccagatgcctttaaaagataaac 1584
gtggtagtagaatttgcttttagcgaaaagctccctatccttgaactcaggatcttcccaaggattgatagc 1656
taacttaatttccacaaaagagtgaaatggattgaccgaagggatagtacaaagttcttcccaaggaatttc 1728
aatcattttcaaaccgtacctttcgcataaccttgaccagttcccaaccaaatcatcgatcaattttggggt 1800
ggtagtcaaccattccaatcttatgtgaaaacagtggtcggggttatgaactctgtcataatggactgtaca 1872
cgattcttgtttcgaggattttcccgcgggatcaacatcgataacaagagagttactcagcattaaaatagg 1944
gcgtgatggctctttggcgtcagcaactgaagcattcgaagcgcttatagcaggtactactgaagaaaaagg 2016
agtcatagcgcttggatcgtttgagctaccagtggatgcttttctcaacatctgctttggatcggataatgt 2088
agagcgatgggagtttgttttttctagtttattagtgtccattacgtattcaggtgagaactggtaaaagta 2160
gtgcccgtccaaaaaattgtgcttatttaaaacgtgaacaaataatccctctttcatcaccttctgcccata 2232
tttaattgcatcttctcga                                                       2251
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Fig. 2. Nucleotide and deduced amino acid sequences of the *HOM6* region The nucleotide sequence is numbered from nucleotide 1 of the presumed initiation codon of *HOM6*.

mized without creating additional gaps between the blocks of similarity.

The *S. cerevisiae* homoserine dehydrogenase is strikingly similar to the last 350 amino acids from both aspartokinase-homoserine dehydrogenases from *E. coli*. This extensive homology encompasses the entire

peptide 1 A L F S N L P T N G F V Y H E A T V G A G L P I * S F L R E

deduced A L F S N **K** P T N G F V Y H E A T V G A G L P I I S F L R E

peptide 2 V Y T N P V * I Q G A G A G A A V T A A G V L G D V I K

deduced **R** Y T N P V V I Q G A G A G A A V T A A G V L G D V I K

Fig 3. Comparison of peptides identified by Yumoto et al. on purified yeast homoserine dehydrogenase with the deduced amino acid sequence of the *HOM6* gene Mismatchs are in bold type; *undetermined

length of the yeast enzyme and starts exactly where the aspartokinase domains of the *E. coli* enzymes end (Fig. 4). 67 amino acid residues are identical between the three enzymes. The *S. cerevisiae* and *E. coli* homoserine dehydrogenases are less homologous to their counterparts from Gram positive bacteria. However, the com-

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ECHDH1  rvl----------KFGGTSVANAERFLRVADILESNARQGQVATVlsapakitnhlvamiektisgqdalpnisdaeri   69
ECHDH2  sviaqagakgrqlhKFGGSSLADVKCYLRVAGIMAEYSQPDDMMVV-saagsttnrliswlklsqtdrlsahqvqqtlrr   79

ECHDH1  faelltglaaaqpgfplaqlktfvdqefaqikhvlhgisllgqcpdsinaalicrgekmsiaimagvlearghnvtvidp  149
ECHDH2  yqcdlisgllpaeeadslisafvsdlerlaalldsgindavy------aevvghgevwsarlmsavlnqqglpaawlda  152

ECHDH1  vekllavghylestvdiaestrriaasripadhmVLMAGFTAGNEKGELVVLGRNGSDYSAAVLAACLRADCCEIWTDVN  229
ECHDH2  reflraeraaqpqvdeglsypllqqllvqhpgkrLVVTGFISRNNAGETVLLGRNGSDYSATQIGALAGVSRVTIWSDVA  232

ECHDH1  GVYTCDPRQVPDARLLKSMSYQEAMELSYFGAKVLHPRTITPIAQFQIpclikntgnpqapgtligasrdedelpvkgis  309
ECHDH2  GVYSADPRKVKDACLLPLLRLDEASELARLAAPVLHARTLQPVSGSEIdlqlrcsytpdqgstriervlasgtgarivts  312

ECHDH1  nlnnmamfsvsgpgmkgmvgmaarvfaamsrarisvvlitqssseysisfcvpqsdcvraeramleefylelkegllepl  389
ECHDH2  hddvcliefqvpasqdfklahkeidqilkraqvrplavgvhndrqllqfcytsevadsalkildeaglpgelrlrqglal  392

ECHDH1  avaerlaiisvvgdglrtlrgisakffaalaraninivaiaqgssersisvvvnnddattgvrvthqmlfntdqvIEVFV  469
ECHDH2  vamvgagvtrnplhchrfwqqlkgqpveftwqsddgislvavlrtgptesliqglhqsvfraekr------- --IGLVL  462
SCHDH   ------------------------------------------------------------------stkvVNVAV    9
BSHDH   ------------------------------------------------------------------ kaIRVGL    7
CGHDH   ---------------------------------------------------------tsasapsfnpkggpgsaVGIAL   22

ECHDH1  IGVGGVGGalleqlkrqqswlknkhidlrvcqvanskalltnvhglnlenwqeelaqakepfnlgrlir lvkeyhllnpv  549
ECHDH2  FGKGNIGSrwlelfareqstlsartgfefvlagvvdssrrsllsydgldasralaffndeaveqdeeslflwmrahpyddl  542
SCHDH   IGAGVVGSafldqllamksitynlvllaeaersliskdfsplnvgsdwkaalaasttktlpl ddliahlktspk---pv  86
BSHDH   LGLGTVGSgvvkiiqdhqdklmhqvgcpvtikkvlvkdlekkrevdlpkevlttcvydviddp dvdvvieviggveqtkq  87
CGHDH   LGFGTVGTevmrlmteygdelahriggplevrgiavsdiskpregvap-elltedafaliere dvdivveviggieypre  101

ECHDH1  i-VNCTSSQAVADQYADFLREGFHVVTPNKKANTSsmdyyhqlryaaeksrrkflyDINVGAGLPVienlqnllnAGDEL  628
ECHDH2  vvLDVTASQQLADQYLDFASHGFHVISANKLAGASdsnkyrqihdafektgrhwlyNATVGAGLPInhtvrdlidSGDTI  622
SCHDH   ilVDNTSSAYIAGFYTKFVENGISIATPNKKAFSSdlatwkalfsnkptng-fvyhEATVGAGLPIisflreiiqTGDEV  165
BSHDH   ylvda-------------LRSKKHVVTANKdlmavygs---ellaeakengcdiyfEASVAGGIPIirtleegl-SSDRI  150
CGHDH   vvlaa-------------LKAGKSVVTANKalvaahsa---eladaaeaanvdlyfEAAVAGAIPVvgplrrsl-AGDQI  164

ECHDH1  MKFSGILSGSLSYIFgkldegms----FSEATRLAREMGYTEPDPRDDLSGMDVARKLLILARetgreleladieiepvl  704
ECHDH2  LSISGIFSGTLSWLFlqfdgsvp----FTELVDQAWQQGLTEPDPRDDLSGKDVSRKLVILAReagyniepdqvrveslv  698
SCHDH   EKIEGIFSGTLSYIFnefstsqandvkFSDVVKVAKKLGYTEPDPRDDLNGLDVARKVTIVGRisgveveseptsfpvqsl  245
BSHDH   TKMMGIVNGTTNFILtkmikeksp---YEEVLKEAQDLGFAEADPTSDVEGLDAARKMAILARlgfsmnVDLFDVKVKGI  227
CGHDH   QSVMGIVNGTTNFIIdamdstqad---YADSLAEATRLGYAEADPTADVEGHDAASKAAILASiafhtrVTADDVYCEGI  241

ECHDH1  -paefnaegDVAAFMANLSQLDDLFAARVAKARDEGKVLRYVGNIDedgvcrvkiaevdgndplfkvkngenalafyshy  783
ECHDH2  -pahce-ggSIDHFFENGDELNFQMVQRLEAAREMGLVLRYVARFDangkarvgveavredhplrsllpcdnvfaiesrw  776
SCHDH   ipkpleSVKSADEFLEKLSDYDKDLTQLKKEAATENKVLRFIGKVDvatksvsvgiekydyshpfaslkgsdnvisiktk  325
BSHDH   SQITDEDISFSKRLGYTMKLIGiaqrdg-----SKIEVSVQPTLLPDHHPLSAVHNEFNAVYVYGEAVGETMFY------  296
CGHDH   SNISAADIEAAQQAGHTIKLLAicekftnkegkSAISARVHPTLLPVSHPLASVNKSFNAIFVEAEAAGRLMFY------  315

ECHDH1  yqplplvlrGYGAGNDVTAAGVFADIlrtlswklgv----------------------------------------------  819
ECHDH2  yrdnplvirGPGAGRDVTAGAIQSDInrlaqll----------------------------------------------  809
SCHDH   rytnpvviqGAGAGAAVTAAGVLGDViklaqrl----------------------------------------------  358
BSHDH   ---------GPGAGSMPTATSVVSDLvavmknmrlgvtgnsfvgpqyeknmkspsdiyaqqfLRIHVKDEVGSFSKITSV  367
CGHDH   ---------GNGAGGAPTASAVLGDVvgaarnkvhggrapgestyanlpiadfgetttryh-LDMDVEDRVGVLAELASL  385

BSHDH   FSERGVSFEKILQlpikghDELAEIVIVTHHTSQADFSDILQNLNDLEVVQEVKSTYRVEgngws  432
CGHDH   FSEQGISLRTIRQeer---DDDARLIVVTHSALESDLSRTVELLKAKPVVKAINSVIRLErd---  444
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Fig. 4. Amino acid sequence comparison of homoserine dehydrogenase. ECHDH1: *E. coli* aspartokinase-homoserine dehydrogenase I [21]; ECHDH2: *E coli* aspartokinase-homoserine dehydrogenase II [22]; SCHDH: *S. cerevisiae* homoserine dehydrogenase, BSHDH: *B subtillis* homoserine dehydrogenase [3]; CG: *C. glutamicum* homoserine dehydrogenase [23]. Blocks of similarity are in upper case letters and boxed. Residues shared by at least three sequences are in bold letters. Black circles indicate the residues strictly conserved between the five proteins.

parison of the five enzymes reveals five significant blocks of similarity. These regions contain the 27 amino acid residues strictly conserved between the five sequences. Among these blocks, the first one contain the motif Gly-X-Gly-X-X-Gly which is found in all known nucleotide binding sites [20]. This region thus might constitute the NAD(P)H binding sites of these dehydrogenases. The two homoserine dehydrogenases from Gram positive bacteria appear to be quite different from the others by containing a C-terminal extension of about 90 amino acid residues strongly conserved between the *B. subtillis* and *C. glutamicum* enzymes. They also lack two insertions of about 15 amino acid residues each shared by the yeast and *E. coli* homoserine dehydrogenases.

4. DISCUSSION

The yeast homoserine dehydrogenase-encoding gene was cloned by functional complementation of a strain bearing a mutated allele at the *HOM6* gene. The yeast homoserine dehydrogenase appears to be composed of 358 amino acid residues which lead to a predicted molecular weight of about 38,000. The *S. cerevisiae* homoserine dehydrogenase thus appears to be the smallest monofunctional homoserine dehydrogenase known to date. Sequence comparisons of microbiological homoserine dehydrogenases reveals that this small size is accounted for by the absence of a C-terminal extension found in homoserine dehydrogenase from Gram positive bacteria. Two kinds of *C. glutamicum* mutants possessing a homoserine dehydrogenase resistant to feedback inhibition by threonine were isolated [1,2]. In the two cases, loss of allosteric inhibition by threonine was associated with a single mutation mapped in the C-terminal extension. *S. cerevisiae* homoserine dehydrogenase was previously reported to be inhibited by L-threonine and L-methionine [9,10]. However, the K_i reported for the inhibition of the purified enzyme by L-threonine and L-methionine were both about 120 mM. This is to be compared to the K_m value for the substrate L-homoserine which is about 1 mM [10]. The cloning of the *HOM6* gene allowed us to measure 6-fold increase of homoserine dehydrogenase activity in strain W303-1A transformed with plasmid pHOM6-3 as compared to the strain without plasmid, in agreement with the *HOM6* gene being expressed from a multicopy plasmid. Furthermore, addition of L-threonine up to 20 mM in the assays did not decrease homoserine dehydrogenase activity in extracts either from the transformed strain or from the parental strain (data not shown). By contrast, the *C. glutamicum* homoserine dehydrogenase activity is 80% inhibited by the addition of 2.5 mM threonine [2]. The *S. cerevisiae* homoserine dehydrogenase is thus a naturally occuring feedback resistant enzyme. Sequence comparisons clearly show that it is to be correlated with the absence of both the C-terminal domain found in Gram positive bacteria and the aspartokinase domain of the Gram negative bacteria.

One can thus speculate that the ancestral homoserine dehydrogenase was a feedback-resistant enzyme, the structural organization of which could have been comparable to that of the *S. cerevisiae* homoserine dehydrogenase. Allosteric inhibition appears to have emerged from two independant gene fusion events that have happened after the divergence between Gram negative and Gram positive bacteria. In Gram negative bacteria, it could have resulted from a direct fusion between aspartokinase and homoserine dehydrogenase as postulated by Parsot and Cohen [3]. By contrast, in gram positive bacteria, allosteric inhibition was gained in a completely different way consisting of the recruitement of an additional domain, the origin of which remains unknown.

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