Evolutionary relationships between yeast and bacterial homoserine dehydrogenases

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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 HindIII digest of chromosomal DNA from strain X2180-1A in the HindIII 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 XhoI–EcoRI 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 XhoI–EcoRI 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 XhoI–HindIII 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 ClaI 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 XhoI–EcoRI 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-
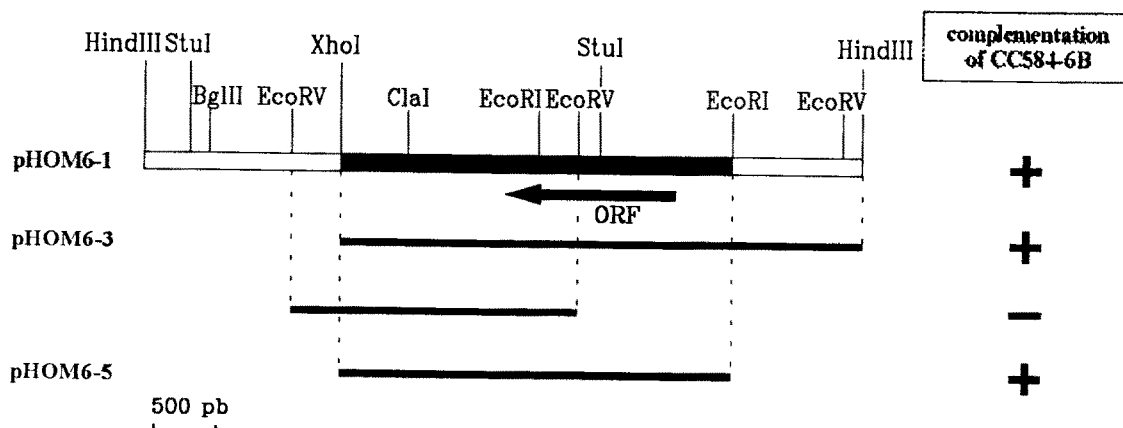

Fig. 1. Physical map of the HOM6 region The fragments subcloned in pEMBLYe23 or pRS316 plasmids, as well as their ability to complement the hom6 mutation of strain CC584-6B are shown.

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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
atttaggatcacttttttgagcatctaatgaagagcttgaagcagaatgaattttcccgattatgtttcccgg  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 strik-ingly similar to the last 350 amino acids from both aspartokinase-homoserine dehydrogenases from E. coli. This extensive homology encompasses the entire

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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
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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  faelltglaaaqpgfplaqlktfvdqefaqikhvlhgisllgqcpdsinaalicrgekmsiaimagvlearghnvtvldp      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   ------------------------------------------------------tsasapsfnpkggpqsaVGIAL       22

ECHDH1  IGVGGVGGalleqlkrqqswlknkhidlrvcqvanskalltnvhglnlenwqeelaqakepfnlgrlir lvkeyhllnpv      549
ECHDH2  FGKGNIGSrwlelfareqstlsartgfefvlagvvdsrrsllsydgldasralaffndeaveqdeeslflwmrahpyddl      542
SCHDH   IGAGVVGSafldqllamkstitynlvllaeaersli skdfsplnvgsdwkaalaasttktlplddliahlktspk---pv       86
BSHDH   LGLGTVGSgvvkiiqdhqdklmhqvgcpvtikkvlvkdlekkrevdlpkevlttcvydviddpdvdvvvieviggveqtkq       87
CGHDH   LGFGTVGTevmrlmteygdelahriggplevrgiavsdiskpregvap-elltedafalieredvdivvveviggieypre      101

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

ECHDH1  MKFSGILSGSLSYIFgkldegms----FSEATRLAREMGYTEPDPRDDLSGMDVARKLLILARetgrel\u2022ladieiepvl      704
ECHDH2  LSISGIFSGTLSWLFlqfdgsvp----FTELVDQAWQOGLTEPDPRDDLSGKDVSRKLVILAReagyni\u2022pdqvrveslv      698
SCHDH   EKIEGIFSGTLSYIFnefstsqandvkFSDVVKVAKKLGYTEPDPRDDLNGLDVARKVTIVGRisgvev\u2022sptsfpvqsl      245
BSHDH   TKMMGIVNGTTNFILtkmikeksp---YEEVLKEAQDLGFAEADPTSDVEGLDAARKMAILARlgfsmnVDLFDVKVKGI      227
CGHDH   QSVMGIVNGTTNFIIdamdstqad---YADSLAEATRLGYAEADPTADVEGHDAASKAAILASiafhtrVTADDVYCEGI      241

ECHDH1  -paefnaegDVAAFMANLSQLDDLFAARVAKARDEGKVLRYVGNIDedgvcrvkia\u2022vdgndplfkvkngenalafyshy      783
ECHDH2  -pahce-ggSIDHFFENGDELNFQMVQRLEAAREMGLVLRYVARFDdvgkaivgveavredhplrsllpcdnvfaiesrw      776
SCHDH   ipkpl\u2022svkSADEFLEKLSDYDKDLTQLKKEAATENKVLRFIGKVDvatksvsvqi\u2022kydyshpfaslkgsdnvisiktk      325
BSHDH   SQITDEDISFSKRLGYTMKLIGLaqrdg-----SKIEVSVQPTLLPDHHPLSAVHNEFNAVYVYGEAVGETMFY-----      296
CGHDH   SNISAADIEAAQQAGHTIKLLAicekftnkegkSAISARVHPTLLPVSHPLASVNKSFNAIFVEAEAAGRLMFY-----      315

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

BSHDH   FSERGVSFEKILQLpikghDELAEIVIVTHHTSQADFSDILQNLNDLEVVQEVKSTYRVEgngws      432
CGHDH   FSEQGISLRTIRQber---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 corre-

lated 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.

REFERENCES

[1] Archer, J.A.C., Solow-Cordero, D.E. and Sinskey, A.J. (1991) Gene 107, 53–59
[2] Reinscheid, D.J., Eikmanns, B.J. and Sahm, H. (1991) J. Bacteriol. 173, 3228–3230
[3] Parsot, C. and Cohen, G.N. (1988) J. Biol. Chem. 263, 14654–14660
[4] Patte, J C., Truffa-Bachi, P and Cohen, G.N (1966) Biochim Biophys. Acta 128, 426–439
[5] Dautry-Varsat, A., Sibili-Weil, L. and Cohen, G.N. (1977) Eur. J Biochem. 76, 1–6
[6] Belfaiza, J., Fazel, A., Müller, K. and Cohen, G.N. (1984) Biochem Biophys. Res. Commun. 123, 16–20.
[7] Truffa-Bachi, P., Veron, M. and Cohen, G.N. (1974) CRC Crit. Rev. Biochem 2, 379–415
[8] Patte, J.C, Le Bras, G and Cohen, G N. (1967) Biochem. Biophys. Acta 136, 245–257
[9] Black, S and Wright, N.G. (1955) J. Biol. Chem. 213, 51–60.
[10] Yumoto, N., Kawata, Y., Noda, S and Tokushige, M. (1991) Archiv. Biochem Biophys. 285, 270–275.
[11] Sherman, F., Fink, G.R. and Hicks, J.B. (1979) Methods in Yeast Genetics· a Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
[12] Ito, H, Fukuda, Y., Murata, K. and Kimura, A. (1983) J. Bacteriol. 153, 163-168.
[13] Baldari, C. and Cesarini, G. (1985) Gene 35, 27–32.
[14] Sikorski, S.R. and Hieter, P (1989) Genetics 122, 19–27.
[15] Ish-Horowicz, D. and Burke, J.F. (1981) Nucleic Acids Res. 9, 2989-2998.
[16] Thomas, D. and Surdin-Kerjan, Y. (1990) Gene Anal. Tech. Appl. 7, 87–90
[17] Dessen, P., Fondrat, C., Valencien, C. and Mugnier, C. (1990) Comp. Appl. Biosci 6, 355-356
[18] Lipman, D J. and Pearson, W.R. (1985) Science 227, 1435-1440.
[19] Schuler, G.D., Altschul, S F., Lipman, D.J. (1991) Proteins Struct. Funct. Genet. 9, 180 190.
[20] Wierenga, R.K., Tepstra, P. and Hol, W.G.J (1986) J. Mol Biol. 187, 101-107.
[21] Cossart, P., Katinka, M., Yaniv, M., Saint-Girons, N. and Cohen, G.N. (1979) Mol. Gen Genet. 175, 39-44.
[22] Zakin, M., Duchange, M., Ferrara, P. and Cohen, G.N. (1983) J. Biol Chem. 258, 3028 3031.
[23] Peoples, O.P., Liebl, W., Bodis, M, Maeng, M.J., Follettie, M.T., Archer, J.A. and Sinskey, A.J. (1988) Mol. Microbiol 2, 63–72.