Short Communication

Molecular Cloning of Rice Lactate Dehydrogenase cDNA

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A full length cDNA clone which encodes rice lactate dehydrogenase (LDH) was isolated from lambda gt11 library and sequenced. The LDH cDNA sequence shows 1026bp of the protein coding region, the 110bp of 5' flanking region and 240bp of 3' flanking region. The primary structure of LDH was deduced from nucleotide sequence. The LDH consists 353 amino acid residues and its molecular mass is deduced to be 38168. In the coding region of cDNA sequence, the rice LDH showed 75% homology with the barley LDHA and 54% with the human LDHB. The alignment of the whole amino acid sequence showed 85% and 45% homologies respectively with those of the barley LDHA and the human LDHB.

KEY WORDS: *Oryza sativa*, lactate dehydrogenase, cDNA cloning

Introduction

In plants the anaerobic response is an adaptive mechanism that enables survival under low oxygen conditions for several days. Plants respond to anaerobic stress by switching from oxidative respiration to fermentative glycolysis. Under hypoxia, protein synthesis ceases while about 20 polypeptides are newly synthesized which are called anaerobic polypeptides (ANPs). Some of the ANPs are enzymes involved in the pathways of anaerobic carbohydrate metabolism, for example alcohol dehydrogenase (DENNIS *et al.* 1984, XIE, Y. and R. WU 1989) and pyruvate decarboxylase (KELLEY *et al.* 1989). The molecular structure and the mechanisms regulating the expression of alcohol dehydrogenase (ADH) have been studied extensively. Similar to ADH, lactate dehydrogenase (LDH) activity in the root of barley increases under anaerobic conditions for up to five days. The increase of the LDH activity has been found to be accompanied by an elevation of mRNA (HONDRED and HANSON, 1989).

Among the cultivated plants, rice is one of the most resistant species to the lack of oxygen. To analyze the molecular mechanisms responsible for the anaerobic regulation of rice LDH, at first we cloned and characterized cDNA encoding rice LDH. We have also compared the deduced amino acid sequence of rice LDH with barley, human, chicken and bacterial LDHs.

Materials and Methods

Plant materials. For the isolation of poly(A)⁺ RNA, rice (*Oryza sativa* L., cv. Nipponbare) and barley (*Hordeum vulgare* L., cv. Carmen) seeds were germinated under dark conditions at 30°C for nine days.

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Preparation of mRNA and construction of cDNA library. Total RNA was isolated from seedlings, and poly(A)⁺ RNA was isolated by using mRNA Purification Kit (Pharmacia). cDNA was synthesized from isolated mRNA, and ligated to EcoRI digested bacteriophage lambda gt11.

Preparation of the probe. Two oligonucleotide primers were synthesized based on barley LDH sequence (HONDRED and HANSON 1989), namely 5'-GACGAGATCGCGC TCGTCGA-3' (nucleotides 258 to 277), and 5'-AATGGCCCAAG AGGTGTACC-3' (nucleotides 857 to 876). The polymerase chain reaction (PCR) amplification was carried out on total barley cDNA library. The 618bp PCR product obtained was used for screening the rice cDNA library.

Isolation of rice LDH cDNA clone and nucleotide sequence analysis. The PCR product was labeled by ECL direct nucleic acid labeling and detection system (Amersham), and was used as a probe for plaque hybridization of the rice cDNA library. Southern transfer was performed by the alkali blotting method (Sambrook *et al.* 1989). The positive inserts were subcloned into pT7T3 phagemid vector (Pharmacia). Single-stranded DNA was isolated through infection of helper phage M13KO7, and was used to determine the nucleotide sequence. Nested deletion clones were produced by using a deletion kit (Takara). The nucleotide sequence was determined by ABI Dyeprimer method.

Results and Discussion

Isolation of LDH cDNA clone. Using the PCR product of 618bp we isolated five putative LDH clones after screening of 120,000 recombinants, and they were further characterized by southern blotting and nucleotide sequencing (Fig. 1). Clones s1, s2 and s4 appear to contain the cDNA insert including the whole coding region and clones s3 and s5 contains shorter cDNA insert.

Nucleotide sequence analysis. The restriction map of the cDNA insert in pT7T3 was constructed, and the sequences were determined according to the strategy shown in Fig. 1. The open reading frame of the nucleotides which corresponds to 353 amino acid residues was identified. The 5' untranslated region is 110bp, and 3' untranslated region is 240bp.

The nucleotide sequence and the deduced amino acid sequence are shown in Fig. 2. The molecular mass of the polypeptide was calculated as 38168. This value is approximately identical with 38K daltons estimated by SDS-polyacrylamide gel electrophoresis of purified rice LDH (date not shown). Nucleotide sequence within the protein coding region was com-

Fig. 1. Physical map and sequencing strategy of rice LDH cDNA. The deletion mutant series obtained by using deletion kit (Takara) was subcloned into the pT7T3 phagemid vector and the nucleotide sequences were determined by the ABI Dyeprimer method. E = *EcoRI*, C = *SacI*, P = *PstI*, S = *SmaI*.

pared with those of barley (HONDRED and HANSON, 1989) and human (SAKAI *et al.* 1987). The rice LDH cDNA shows a 75% homology with the barley LDHA, and 54% with the human LDHB (data not shown). There is a very high G/C content (70%) in coding region of rice LDH comparing with vertebrate and bacterial LDH.

Deduced amino acid sequence analysis. We compared the deduced amino acid sequence of rice LDH with those of barley, human, chicken and bacterial LDHs. The size difference is largely attributable to variations in the length of the N-terminal extensions. Fig. 3 shows the

1	TC CAT TTC AGG AGA GAT CCA CAT CGC CGT TGG CCT TTC ATC CAG TGA GTG AGG GAG GGA GAT CTC GAT CCA GTG	74
75	AGT GAG GGA GGG AGA TCT CGA TCT CGG TCT CGC GAC ATG AAG AAG GCT TCG TCT CTG TCG GAG CTG GGG TTC GAC	149
1	Met Lys Lys Ala Ser Ser Leu Ser Glu Leu Gly Phe Asp	13
150	GCG GAG GGC GCG TCG TCG GGG TTC TTC CGT CCG GTG GCG GAC GGC GGG TCG ACG CCG ACG TCG CAC CGG CGT CGG	224
14	Ala Glu Gly Ala Ser Ser Gly Phe Phe Arg Pro Val Ala Asp Gly Gly Ser Thr Pro Thr Ser His Arg Arg Arg	38
225	CTG ACG AAG ATA TCG GTG ATC GGC GCG GGC AAC GTG GGG ATG GCG ATC GCG CAG ACC ATC CTG ACC CGG GAC ATG	299
39	Leu Thr Lys Ile Ser Val Ile Gly Ala Gly Asn Val Gly Met Ala Ile Ala Gln Thr Ile Leu Thr Arg Asp Met	63
300	GCG GAC GAG ATC GCG CTG GTG GAC GCG GTG CCG GAC AAG CTG CGC GGG GAG ATG CTG GAC CTG CAG CAC GCG GCG	374
64	Ala Asp Glu Ile Ala Leu Val Asp Ala Val Pro Asp Lys Leu Arg Gly Glu Met Leu Asp Leu Gln His Ala Ala	88
375	GCG TTC CTC CCC CGC GTC CGC CTC GTC TCC GAC ACC GAC CTG GCC GTC ACG CGC GGC TCC GAC CTG GCC ATC GTC	449
89	Ala Phe Leu Pro Arg Val Arg Leu Val Ser Asp Thr Asp Leu Ala Val Thr Arg Gly Ser Asp Leu Ala Ile Val	113
450	ACG GCC GGC GCG CGC CAG ATC CCC GGG GAG AGC CGC CTG AAC CTG CTG CAG CGG AAC GTG GCG CTG TTC CGG AAG	524
114	Thr Ala Gly Ala Arg Gln Ile Pro Gly Glu Ser Arg Leu Asn Leu Leu Gln Arg Asn Val Ala Leu Phe Arg Lys	138
525	ATC GTG CCG GCG CTG GCG GAG CAC TCG CCG GAG GCG CTG CTG CTG ATC GTC TCC AAC CCC GTC GAC GTG CTG ACG	599
139	Ile Val Pro Ala Leu Ala Glu His Ser Pro Glu Ala Leu Leu Leu Ile Val Ser Asn Pro Val Asp Val Leu Thr	163
600	TAC GTG GCG TGG AAG CTG TCG GGG TTC CCG GCG AGC CGC GTC ATC GGC TCC GGC ACC AAC CTC GAC TCC TCC AGG	674
164	Tyr Val Ala Trp Lys Leu Ser Gly Phe Pro Ala Ser Arg Val Ile Gly Ser Gly Thr Asn Leu Asp Ser Ser Arg	188
675	TTC CGC TTC CTC CTC GCC GAG CAC CTC CAG GTC AAC GCC CAG GAT GTC CAG GCG TAC ATG GTG GGA GAG CAC GGG	749
189	Phe Arg Phe Leu Leu Ala Glu His Leu Gln Val Asn Ala Gln Asp Val Gln Ala Tyr Met Val Gly Glu His Gly	213
750	GAC AGC TCG GTG GCG ATA TGG TCG AGC ATG AGC GTG GCC GGG ATG CCG GTG CTC AAG TCG CTG CGG GAG AGC CAC	824
214	Asp Ser Ser Val Ala Ile Trp Ser Ser Met Ser Val Ala Gly Met Pro Val Leu Lys Ser Leu Arg Glu Ser His	238
825	CAG AGC TTC GAC GAG GAG GCC CTG GAG GGA ATC CGG CGA GCG GTG GTG GAC AGC GCG TAC GAG GTG ATC AGC CTC	899
239	Gln Ser Phe Asp Glu Glu Ala Leu Glu Gly Ile Arg Arg Ala Val Val Asp Ser Ala Tyr Glu Val Ile Ser Leu	263
900	AAG GGC TAC ACC TCC TGG GCC ATC GGC TAC TCC GTC GCC AGC CTC GCC GCC TCC CTC CTC CGC GAC CAG CAC CGC	974
264	Lys Gly Tyr Thr Ser Trp Ala Ile Gly Tyr Ser Val Ala Ser Leu Ala Ala Ser Leu Leu Arg Asp Gln His Arg	288
975	ATC CAC CCC GTC TCC GTC CTC GCC TCC GGC TTC CAC GGC ATC CCC CAA GAC CAC GAG GTC TTC CTC AGC CTC CCC	1049
289	Ile His Pro Val Ser Val Leu Ala Ser Gly Phe His Gly Ile Pro Gln Asp His Glu Val Phe Leu Ser Leu Pro	313
1050	GCC CGC CTC GGC CGC GCC GGC GTC CTC GGC GTC GCC GAG ATG GAG CTC ACC GAG GAG GAG GCC CGC CGC CTC CGC	1124
314	Ala Arg Leu Gly Arg Ala Gly Val Leu Gly Val Ala Glu Met Glu Leu Thr Glu Glu Glu Ala Arg Arg Leu Arg	338
1125	CGC TCC GCC AAG ACG CTC TGG GAG AAC TGC CAG CTG CTC GAC CTC TAA TAA ATC TTA TTA TTA TCA TCG TCG TCG	1199
339	Arg Ser Ala Lys Thr Leu Trp Glu Asn Cys Gln Leu Leu Asp Leu ***	354
1200	TCG TCT CGT CAC GGA ATT AAT TAA AGT ACC TAC TCC GTA CTT AGC TAG CTA CAA TAA TAA GGA TTC ATT GAT CAC	1274
1275	TAC AAG AGT GAT CGA CTC GAC TGT AGT ATG TGT GTG CAA TAT AAT GTG CTG TCT ATC AAC AAC TAC TAG TAT TGT	1349
1350	CAT TTT TTT CGA ACC AGG GAA CTT TTT AAT GAT AAG AAG AAA AAG ACA AGT ACT TAT TGT CGA	1412

Fig. 2. Nucleotid and amino acid sequences of rice LDH. The deduced amino acid sequence of rice LDH is indicated below the nucleotide sequence.

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      20              40              60              80
MKKASSLSELGFDAEGASSGFFRPVADGGS TPTS HRRRLTKISVIGAGNVGMAIAQTILTRQMADEIALVDAVPD
*H*****G*****CPA*****SAVP*** **QNL*****L**
      MATLKEKLI*VAEEEA *VPNN          **V*V*V*Q***C*IS**GKSL***L***VLE*
      SLK*HLIHNHVKEEHA*HNKISSVGV***A***C*IS**MK*L***LT***V*E*
      MKNNGGARVV*****F**ASYVFALMNQGI****V*I**NES
      100              120              140              160
KLRGEMLDLQHAAFLPRVRLVSDTFLAVTRGSDLAIVTAGARQIPGESRLNLLQRNVALFRKIVPALAEHSPEALLLIV
*****I *G**A***KN***V*****T*****Y*****PV*****D***V*
**K**M***GSL**QTPKI*ADK*YS**AN*KIVV*****QE*****V***NV*KF*I*QTVKV**DCIIIV*
**K**M***GSL**KTPKIT*GK*YS**AN*KV*****QE*****V***NV*KF*I*NVVKV**DCK**I*
*AI*DAM*FNHGKV*A*KPVDIWHG*YDDC*EAELVIC***NKP**T**D*VDK*I*D**S**SEVMAHGFQG*F*VA
      180              200              220              240
SNPVDVLTVAWKLSGFPASRVIGSGTNLDSSRFRFLLAEHLQVNAQDVQAYMVGEHGDSSVAIWSSMSVAGMPVLKSLR
*****E*****I****D*****I**G***AF***
*****I****I*****L*KH*****C****A***Y*M**K*GIHPSSCHGWIL*****V**GVN***VSLQELNP
*****I*****KH*****C****A***H*MG*R*GIHPLSCHGWI**Q*****PV**GVN***VSLKAIHP
T***D***AT**F**L*HE*****TI**TA*****G*YFS*AP*NVH**II*****TELPV**QAYIGVM*IR* LV
      260              280              300              320
ESHQSFDEEALLEGIRRAVDSAVEVISLKGYSWAIGYSVASLAASLLRDQHRHFPVSVLASGFHGIPQDHEVFLSLPAR
D**R***A*****GG*****G*****R**V*****SDG*****
**MGTDN*S*NWKEYHKW**E*S***K*****L***D*IE*M*KNLS*****TAVK*MY**EN *****CI
DMGTD*A*K*NWKEYHKQ**D*****K*****L***D**ETIMKNLR*V**I*TAVK*MH**KD* *****V*CV
**KGEEAQKI**R*FVN*R*A**QI*EK**A*YYG*AMGL*RVTRAI*HNENA*LTV*AYLD*LY*ERD *YIGV**V
      340
LGRAGVLGVAEMELTEEEARRLRSAKTLWENCQLLDL RICE LDH
**G*I*****D***A**AQ***** BARLEY LDHA
*NAR*LTS*INQK*KDD*VAQ*KK**D***DIQKD*KD HUMAN LDHB
**SS*ITD*VK*I*KPD*EEKIKK**D***GIQKE*QF CHICKEN LDHM
IN*N*IRE*I*I**NDD*KN*FHH**A**KSVLARAFTR Bacillus stearothermophilus LDH

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Fig. 3. Comparison of the whole amino acid sequence of LDHs. The sequence of rice LDH is shown and compared with those of barley LDHA, human LDHB, chicken LDH M and bacterial LDH. Identical amino acids with rice LDH are shown by asterisks. Gaps, attributed to deletions or insertions, is indicated blank spaces.

five sequences aligned together to maximum homology. The rice LDH amino acid sequence has high homology with the barley LDHA (85%), and shows 45% homology with human LDHB and chicken LDH M, 34% homology with bacterial LDH. Several regions which are well conserved in all the five species were found. Among them residue 113~138 and 161~197 correspond to loop region and substrate binding region, respectively (Li, *et al.* 1983).

LDH from various sources are usually works as tetramers that exists as a set of five isozymes, which are produced by random association of two subunit types encoded by two separate genes. We tried to screen another rice LDH gene from rice cDNA library by using s4 insert as a probe, but five clones isolated and partially sequenced were same to the s4. These findings suggest that in the seedlings, one of the LDH genes is predominately expressed or is much stable than others because we detected multiple bands by genomic southern hybridization (data not shown). The rice LDH cDNA clone will make it possible to study the molecular mechanism of the anaerobic regulation.

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イネにおける乳酸脱水素酵素cDNAのクローニング

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オオムギ乳酸脱水素酵素(LDH)cDNAの1部分をPCR法によって合成した。これをプローブに用いて、イネcDNAライブラリーをスクリーニングし、イネLDHcDNAをクローニングし、塩基配列を決定した。cDNAクローンは1412 bpで、1062 bpのタンパク質翻訳領域、1102 bpの5'非翻訳領域、および240 bpの3'非翻訳領域からなる。イネLDHの一次構造がcDNA配列から推定された。そのアミノ酸配列は353残基で、分子量は38168ダルトンと計算された。タンパク質翻訳領域におけるイネcDNAの塩基配列は、オオムギおよびヒトとそれぞれ75%、54%の相同性を示した。また、アミノ酸配列の相同性は、オオムギおよびヒトとそれぞれ85%および45%であった。

The nucleotide sequence data reported in this paper will appear in DDBJ, EMBL and GenBank Nucleotide Sequence Databases with the following accession number D13817.