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A rice protein library: a data-file of rice proteins separated by two-dimensional electrophoresis

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Abstract. Proteins extracted from embryos, endosperms and leaves of rice were separated by two-dimensional electrophoresis and relative molecular weights and isoelectric points were determined. The separated proteins were electroblotted onto a polyvinylidene difluoride membrane and 85 electroblotted proteins were analyzed by a gas-phase protein sequencer. The N-terminal amino-acid sequences of 27 out of 85 proteins were determined in this manner. The N-terminal regions of the remaining proteins could not be sequenced and they were inferred to have a blocking group at the N-terminus. Among proteins, 11 could be sequenced after deblocking by in situ treatment with pyroglutamyl peptidase. The internal amino-acid sequences of 23 proteins were determined by sequence analysis of peptides obtained by Cleveland peptide mapping. The amino-acid sequences determined here were compared with those of known plant and animal proteins. The concanavalin A-peroxidase method was used to determine whether the 85 proteins were glycosylated and the diagonal electrophoresis method was used to determine whether they contained disulphide bonding. Finally, we constructed a data-file of rice proteins including information on relative molecular weight, isoelectric point, amino-acid sequence, sequence homology, glycosylation, and the presence of disulphide bonding.

Key words: Rice – Embryo proteins – Endosperm proteins – Leaf proteins – Protein database

Introduction

High-resolution two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is very useful for separating complex protein mixtures (O'Farrell 1975). In conjunction with automated gel scanning and computer-assisted analysis, 2D-PAGE has contributed to the development of protein databases (Anderson and Anderson 1977; Celis et al. 1989; Garrels and Franza 1989).

In 1987, we initiated a study on the construction of a data-file on rice proteins. In the present study, a large number of rice proteins was separated by 2D-PAGE and electroblotted onto a polyvinylidene difluoride (PVDF) membrane to determine their biochemical and physicochemical characteristics, including partial amino-acid sequences. The data obtained were complied as a data-file.

One major advantage of a rice 2D-PAGE database in which most known proteins are identified is the wealth of new proteins that will become amenable to exprimentation both at the biochemical and molecular levels. Accordingly, a high-priority goal in our program has been to establish a database of partial sequences of unknown proteins that may be interfaced with the forthcoming DNA sequence information from the Rice Genome Project. Besides aiding in the identification of known proteins, these sequences can be used to prepare oligodeoxyribonucleotides which, in turn, are used to clone the corresponding cDNAs.

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A part of the rice protein data-file constructed so far is presented, and a strategy to overcome problems faced during the construction of this data-file is discussed.

Materials and methods

Plant materials

Mature seeds and leaves of rice (*Oryza sativa* L.) cultivars Nipponbare and Norin 29, and a semidwarf line SC-TN 1 (Kikuchi et al. 1985) were used.

Gel electrophoresis

A portion (10 mg) of endosperms and embryos from dry mature seeds and leaves at 20 days following germination were removed, homogenized with 100 µl of lysis buffer (O'Farrell 1975) and centrifuged at 15,000 g for 5 min. The supernatant was subjected to 2D-PAGE, as described by Hirano (1982). Sodium dodecyl sulphate (SDS)-PAGE of the total endosperm protein was performed with 15% separation gels and 5% stacking gels. The isoelectric point and molecular weight of each protein were determined using a Pharmacia LKB kit (Uppsala, Sweden).

N-terminal amino-acid sequence analysis

Following separation by 2D-PAGE, the proteins were electroblotted onto a PVDF membrane (ProBlott, Applied Biosystems, Foster City) and detected by Ponceau 3R staining (Hirano 1989). They were cut out from the PVDF membrane and applied to the upper glass block of the reaction chamber in a gas-phase protein sequencer (477A, Applied Biosystems). Edman degradation was performed according to the standard programme supplied by Applied Biosystems. The released phenylthiohydantoin amino-acid derivatives were identified by the on-line system of high-performance liquid chromatography (120A, Applied Biosystems).

Removal of blocked N-terminal amino acids

After 2D-PAGE, the proteins possessing a blocked N-terminal amino acid were electroblotted onto the PVDF membrane and allowed to react with pyroglutamyl peptidase according to the

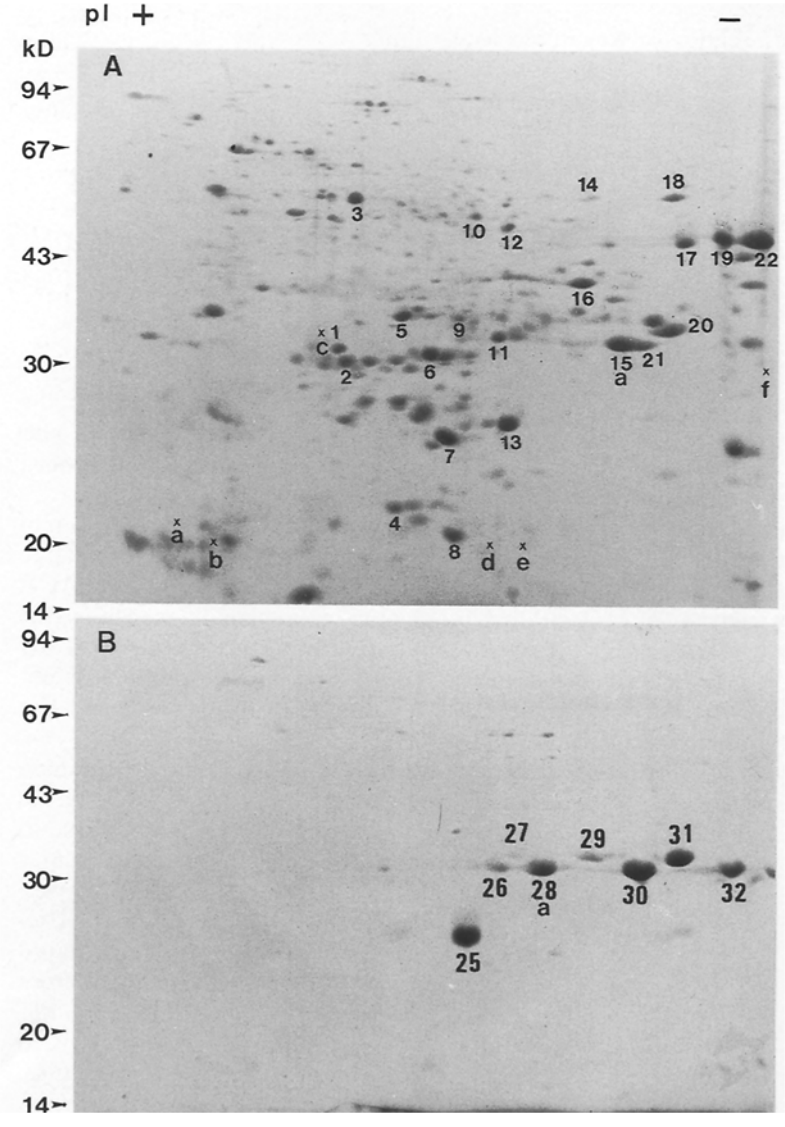

Fig. 1A, B. 2D-PAGE patterns of seed embryo and endosperm proteins from rice (cv Nipponbare). Proteins were extracted from seed embryos (**A**) and endosperms (**B**) of rice. The proteins separated by 2D-PAGE were detected by Coomassie blue staining. Right to left: isoelectric focussing for the first dimension; top to bottom: SDS-PAGE for the second dimension. Molecular weight marker-proteins: *14*, alpha-lactalbumin (Mr 14.4 kDa); *20*, trypsin inhibitor (20.1 kDa); *30*, carbonic anhydrase 30.0 kDa); *43*, ovalbumin (43.0 kDa); *67*, albumin (67.0 kDa); *94*, phosphorylase *b* (94.0 kDa). Isoelectric point marker-proteins: *a*, soybean trypsin inhibitor (4.55/20 kDa); *b*, beta-lactoglobulin A (5.20/18 kDa); *c*, bovine carbonic anhydrase B (5.85/30 kDa); *d*, horse myoglobin (6.85/16 kDa); *e*, horse myoglobin (7.35/16 kDa); *f*, lentil lectin (8.15/30 kDa)

method of Hirano et al. (1991) to remove N-terminal pyroglutamic acid. After digestion, proteins on the membrane were subjected to gas-phase sequencing.

Internal amino-acid sequence analysis

The proteins were separated by 2D-PAGE and stained with Coomassie Brilliant blue R250. Gel pieces containing protein spots were removed and the protein was electroeluted from the gel pieces using an Electrophoretic Concentrator (M1759, ISCO, Lincoln) at 2 W constant power for 2 h. After electroelution, the protein solution was dialyzed against deionized water for 2 days and lyophilized. The protein was dissolved in 20 µl of SDS sample buffer (pH 6.8) and applied to a sample well in an SDS-PAGE gel. Twenty microliters of a solution containing 10 µl of *Staphylococcus aureus* V8 protease (Pierce, Rockford) (0.1 µg/µl) in deionized water and 10 µl of SDS sample buffer (pH 6.8) were overlaid on the sample solution. Electrophoresis was performed until the sample and protease were stacked in the upper gel, and interrupted for 30 min to digest the protein (Cleveland et al. 1977). Electrophoresis was then continued and the separated digests were electroblotted on the PVDF membrane and subjected to gas-phase sequencing (Hirano and Watanabe 1990).

Homology search of amino-acid sequences

The amino-acid sequences obtained were compared with those of 7,967 proteins in the amino-acid sequence data base (National Biomedical Research Foundation, Protein Identification Resource; Release 28.0, 1991).

Detection of glycoprotein with an N-linked oligosaccharide chain and disulphide bonding

Proteins of the embryo and endosperm were separated by SDS-PAGE, electroblotted onto a PVDF membrane and reacted with peroxidase-coupled concanavalin A (Honen, Tokyo) according to the procedure of Kijimoto-Ochiai et al. (1985).

Proteins of the embryo and endosperm were extracted in SDS sample buffer (Laemmli 1970), pH 6.8, without 2-mercaptoethanol (Lawrence and Shepherd 1980) and separated by SDS-PAGE using disk gels in the first dimension. The gel was removed from the tube and incubated for 30 min in SDS sample buffer, pH 6.8, containing 1% 2-mercaptoethanol for protein reduction. The disk gel containing the proteins was subjected to SDS-PAGE in the second dimension (Singh and Shepherd 1985) to identify disulphide bonding.

Results and discussion

Two-dimensional electrophoresis patterns of rice proteins

The proteins were separated from seed embryos, endosperms and leaves of rice by 2D-PAGE and detected by Coomassie blue or silver staining. More than 600 and 100 major protein spots were identified by Coomassie blue staining in 2D-PAGE patterns of the embryos and endosperms, respectively (Fig. 1), and more than 150 and 700 spots by Coomassie blue and silver staining, respectively, in that of leaves (Fig. 2).

In 2D-PAGE, the proteins often showed slightly different electrophoretic mobility according to the gel, possibly due to differences in gel conditions such as concentrations of acrylamide and methylene-bis-acrylamide, pH and ionic strength of gel buffers, and temperature of the gel during electrophoresis. The patterns may also be affected by compounds

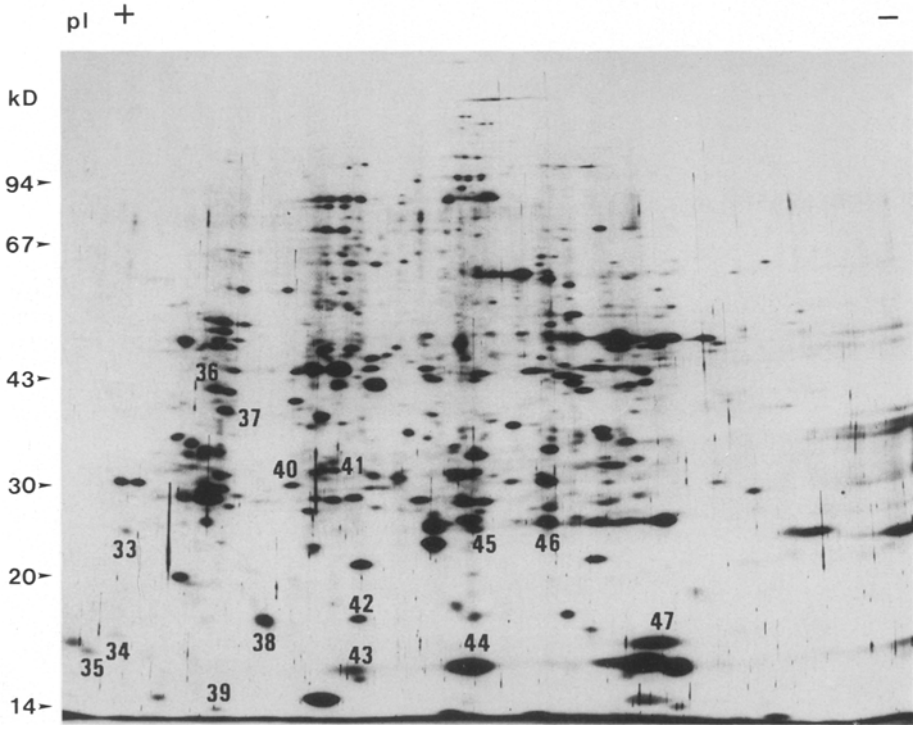

Fig. 2. 2D-PAGE patterns of leaf proteins from rice (cv Nipponbare). Proteins were extracted from leaves of rice 25 days after germination. The proteins separated by 2D-PAGE were detected by silver staining. Right to left: isoelectric focusing for the first dimension; top to bottom: SDS-PAGE for the second dimension

Table 1. Rice protein data-file

No.	Variety	Tissue	pI	MW (kDa)	Homologous protein	Homology (%)	Glycosylation	Disulphide bonding	Sequence	
									N-terminal	internal
1	Nipponbare	Embryo	5.9	32	α-Glucan branching enzyme	63		+	EAAAGPFNILGQ	
2	Norin 29	Embryo	5.9	31	Hemaglutinin precursor	60		+	GPKFVVGGNLKKLGSKDTVDKI	
3	Norin 29	Embryo	6.0	60					SGAILAGVTTXAAN	
4	Nipponbare	Embryo	6.3	22	UDP-glucose hydrolase	63			SFVAVTVYRITTFY	LAAMAGYAKEFDKRGVKL
5	Nipponbare	Embryo	6.3	40						AIKQLNMVDP
6	Norin 29	Embryo	6.5	31						VAGAYGGAIDILV
7	Norin 29	Embryo	6.7	27						YGNPVTGA
8	Nipponbare	Embryo	6.7	20				+	WSGGGEF	IMHP
										VXRPWLXXNKXXL
9	Nipponbare	Embryo	6.8	36						MEXPIKXXDN
10	Nipponbare	Embryo	6.8	57						
11	Nipponbare	Embryo	7.2	32				+		
12	Nipponbare	Embryo	7.2	55						
13	Nipponbare	Embryo	7.3	27	Acyl-CoA oxidase	53			PGLTIGDIVPNLGLD	LAAMAGYAKEFDKRGVKLLGKH
					H^+-transporting ATP-synthase	55				AIKQLNMVDPEKDSN
										MGLTIGDTVX
14	Nipponbare	Embryo	7.4	60						
15a	Nipponbare	Embryo	7.4	32	(SRP-1) glutelin	100	+		⟨QQLLGQSTSQWQSXRRG	FFDVSNEQFQXTGVSAVRRVIEPGLLL
										SYQQQ
	Norin 29	Embryo	7.4	32						SQSQSQKFKDEHQKIHRFRQGDVI
15b	SC-TN1	Embryo	7.7	32	(SRP-2) glutelin	100	+		⟨QQLLGQSTSQ	FFDVSNEQFQXTGVSAVRRVIEPRGLLL
										SYQQQFQQSGQALTESQSQSHKFKDEH-
16	Nipponbare	Embryo	7.4	48	Phospholipase A2	42				QKIHRFRQGDVIALPAG
17	Nipponbare	Embryo	7.4	52			+			TADASDDKLGEYKDY
18	Nipponbare	Embryo	7.7	60						
19	Nipponbare	Embryo	7.7	54	α-Amylase	71	+		WSPPGLWLDEDLLR	SVIVIPAEEPDE
20	Nipponbare	Embryo	7.7	36	NADH dehydro-genase	50				SVLWTESRDVGGGFNNIIVXNIYLN
										SVDLTEGW GNVVLHPTNX
21	Nipponbare	Embryo	7.5	32	Primase	57		+		AGYRHVDTAAQYGIEQXXGI
22	Nipponbare	Embryo	7.8	53	BSRF		+		WSPPGLWLDEDLLR	SVIVIPAEEPDE
23	Norin 29	Embryo			Glutelin II precursor	67				SQSQ
										HQSQHRFRQDGVXAL
24	Norin 29	Embryo							HPEAIPQ	
25	Nipponbare	Endosperm	7.0	26	(Globulin)		−	+	⟨QLSESEMRFRD	RELFAFQQQLQVQL
					Wheat HMW glutenin	73				SMPP
										GYYGEQQQQPG
26	Norin 29	Endosperm	7.2	32	(Glutelin)				⟨QLLXQSTSQSQSXRXGSP	SQSQ
27	Norin 29	Endosperm	7.3	31	(Glutelin)				⟨QLLXQSTSQWQ	SQSQSQKFKDEXQK
28a	Norin 29	Endosperm	7.4	32	(Glutelin)		+		⟨QQLLGQSTSQWQ	FFDVSNEQFQXTGVSAVRRVIEPRGLL
										SQSQSQKFKDEHQKIXXFRQG

	Nipponbare	Endosperm	7.7	32				
28b.	SC-TN1	Endosperm	7.7	32	(Glutelin)	+	‹QQLLGQSTSQWQSS	FFDVSNEQFQXTGVSAVRRVIEPRGL SQSQSH
29	Norin 29	Endosperm	7.4	32	(Glutelin)	+	‹QQLLGQSTSQWQSS	LFQXTGVSVVRRVIEPRGLLL SQSQSQKFKDEHQKIH- XFXQGDXIALALPA
	Nipponbare	Endosperm	7.4	32				
	SN-TN1	Endosperm	7.4	32				
30	Norin 29	Endosperm	7.7	32	(Glutelin)	+	‹QQLLGQSTSQWQSSRR	LFQXTGVSVVRRVIE SQSQSHKFKDEHQKIHRFRQGDVIAL- PAG
	Nipponbare	Endosperm	7.7	32				
	SN-TN1	Endosperm	7.7	32				
31	Norin 29	Endosperm	7.8	31	(Glutelin)	+	‹QQLLGQSTSQWQSSRRGSXRG	LFQXTGVSVVRRXIE TYQQQFQQSGQAQLTES- QSQSHKFKDEHQKIH- RFRQGDVIALPAGVA
	Nipponbare	Endosperm	7.8	31				
	SN-TN1	Endosperm	7.8	31				
32	Norin 29	Endosperm	8.0	31	(Glutelin)	+	‹QLFGPNVNPWHNPRQGGFRE	YFDEKNEQFQXTGTFVIRRVIEP- QGLLVPRYSNTPGMVYIIQ FLLAGNNNREQQMYGRS
	Nipponbare	Endosperm	8.0	31				
	SN-TN1	Endosperm	8.0	31				
33	Nippinbare	Leaf	3.5	22	Parvalbumin		AGGVDDAPLVGNKAPDFF- AEEVFFQQFINV	
34	Nipponbare	Leaf	4.0	16			AVVAAPGAGG	
35	Nipponbare	Leaf	4.0	15	Phospholipase A2		AVVAAPGAGGAGAAADEAP- AVKTLFD AAADEAPAVKTLFD	
36	Nipponbare	Leaf	4.5	45			AVQQDAAV	
37	Nipponbare	Leaf	4.8	40	H_2 class I		WAPAVVV	
38	Nipponbare	Leaf	5.0	16			AGIXLVA	
39	Nipponbare	Leaf	5.0	14	Plastocyanin		QEVLLGAGANGVLVFEPNXFTV	
40	Nipponbare	Leaf	5.4	35			AGVVKFFYGEAE	
41	Nipponbare	Leaf	5.7	35			ATVVWPKYELL	
42	Nipponbare	Leaf	5.7	16	SOD		ATKKAVAVLIGTHQVEGVVV	
43	Nipponbare	Leaf	6.0	15			HTIIHVHVLXG	
44	Nipponbare	Leaf	6.3	14	RuBisCo/SS		IQVWPIEGIKKFELTSYLPPLTV	
45	Nipponbare	Leaf	6.3	25	Acelolactate synthetase isomerase I		ALNYPVVSAEYQEAVEKA	
46	Nipponbare	Leaf	7.0	25			VKAVAV	
47	Nipponbare	Leaf	7.5	14	RuBisCo/SS		XQVWPIEGIKKFELTSYLP	
48	Nipponbare	Leaf					SEVLLGANGGMLVFEPPGF	
49	Nipponbare	Leaf			SOD		ATKKAVAVLKGTHQVEGS	
50	Nipponbare	Leaf					WVTHGXYYGVAPW	

BSRF = Hypothetical BSRF1 protein; wheat HMW glutenin = high-molecular-weight subunit of the glutenin in wheat; SOD = superoxide dismutase; RuBisCo/ss = small subunit of ribulose-1,5-bisphosphate carboxylase

such as salt and polyphenol in particular tissues when crude protein extracts are directly electrophoresed. The electrophoresis patterns for different gels or different tissues could thus not be easily compared without standardization of the electrophoresis patterns.

In this study, protein patterns on gels were standardized by an image analyzer, making reference to molecular weight and isoelectric point marker-proteins. Following pattern standardization, it was possible to determine whether the same protein could be identified as the same spot between gels or among embryos, endosperms and leaves. For example, it was proven by electrophoresis pattern standardization that spot 15a in the embryos was identical to spot 28a protein in the endosperms. This was confirmed by partial amino-acid sequence analysis of the proteins. The image analysis system permitted efficient standardization in a short time without being in any way subjective.

A total of eight glutelin alpha-subunits was identified by 2D-PAGE. The amino-acid sequences in the N-terminal and internal regions of these proteins were determined. They were highly homologous with the sequences of glutelin alpha-subunits deduced from cDNAs. The sequences in this manuscript were thus considered not to be due to contaminants.

N-terminal amino-acid sequence analysis

The proteins separated by 2D-PAGE were electroblotted from gels onto PVDF membranes and visualized by Coomassie blue staining. The protein spots were cut out from the membrane to be sequenced directly on a gas-phase protein sequencer. Eighty-five protein spots from the embryos, endosperms and leaves were subjected to N-terminal sequence analysis. The N-terminal amino-acid sequences of 27 out of 85 proteins were determined as shown in Table 1. Since all 85 proteins appeared at sufficient amounts (possibly more than 100 pmoles) for sequencing on the basis of Coomassie blue staining intensity, most of them (about 70%) were considered to have a blocking group at the N-terminus.

Amino-acid sequence analysis of blocked proteins

About 70% of the proteins were deduced to have a blocking group at the N-terminus. In such cases, the proteins could not be sequenced without removing the group or the N-terminally blocked amino acid.

The potentially blocked electroblotted proteins were digested on PVDF membranes with pyrogutamyl peptidase and subjected to N-terminal sequence analysis by the gas-phase protein sequencer. The amino-acid sequences from the second residue of 11 proteins were determined (Table 1). The N-terminal amino acid of each of the proteins was considered to be pyroglutamic acid formed by the cyclization of glutamine or glutamic acid.

Recently, the authors have developed a sequential deblocking technique for proteins electroblotted on PVDF membranes, whose N-terminal formyl groups, pyroglutamic acids, or acetyl amino acids, can be effectively removed from N-terminally blocked proteins using the same samples on the membrane. It should be possible to analyze other blocked proteins by this sequential deblocking technique, thus enlarging the protein data-file.

Internal amino-acid sequences of proteins

To obtain detailed sequence information necessary for cloning and characterization of the DNA encoding the protein, it is desirable to elucidate the amino-acid sequences not only of the N-terminal region but of the internal region of the protein as well. If proteins having a blocked terminal amino group cannot be deblocked by the above method, it is important to sequence the internal regions of the protein. The Cleveland mapping technique was used to determine the amino-acid sequences of the internal regions of proteins. This method is both relatively easy to use and sufficiently sensitive for this purpose.

Internal stretches of sequences were determined for 58 proteins, including 38 species whose N-terminal amino-acid sequences were determined by the Cleveland method using digestion with *S. aureus* V8 protease. The internal amino-acid sequences of 23 proteins were determined (Table 1).

Homology search of amino-acid sequences

The amino-acid sequences were compared with those of the PIR data-base (Table 1). The amino-acid sequences of 14 proteins were found to be identical to those of proteins already reported. These proteins include the major seed storage protein glutelins (Takaiwa et al. 1987), alpha-amylase (Rogers and Milliman 1983) and NADH-dehydrogenase (Bibb et al. 1981). Proteins highly homologous to those from other plants, such as wheat glutenin (Sugiyama et al. 1985), parvalbumin (Jauregui-Adell and Pechere 1978), superoxide dismutase (Kanematsu and Asada 1990), the small subunit of ribulose-1, 5-bisphosphate carboxylase (Matsuoka et al. 1988), and plastocyanin (Yano et al. 1989), were also identified. On the other hand, 19 proteins had identical partial amino-acid sequences to proteins from animals, such as the hemaglutinin precursor (Chamber et al. 1989) and phospholipase A2 (Kondo et al. 1989).

Spot 28a (Norin 29 and Nipponbare) and spot 28b (SC-TN1) in endosperm (Table 1), detected as proteins with different isoelectric points, 7.4 and 7.7 respective-

ly, but with an identical molecular weight, 37 kDa, by 2D-PAGE, have been identified as semidwarfism-related proteins (Hirano et al. 1991; Nakamura et al. 1991). These proteins have the same N-terminal and internal amino-acid sequences, highly homologous with those deduced from the nucleotide sequence of type I cDNA encoding the glutelin seed storage proteins (Takaiwa et al. 1987).

Glycosylation and disulphide bond

After being electroblotted on the PVDF membranes, the embryo and endosperm proteins were examined by the concanavalin A-peroxidase method to determine whether they possessed N-linked glycosylation sites. Three and six proteins were identified as glycoproteins in embryos and endosperms, respectively.

Total proteins from the embryos or endosperms were separated by diagonal electrophoresis under non-reducing conditions in the first dimension and reducing conditions in the second dimension to determine whether they had inter-disulphide or intra-disulphide bonding. The diagonal electrophoresis patterns of rice seed embryo and endosperm proteins were analyzed. Five proteins of rice embryos were identified as proteins having disulphide bonds. One protein of rice endosperm also possessed a disulphide bond.

Protein databases

The results of micro-characterization of proteins separated by 2D-PAGE were compiled to obtain a data-file, appropriately designated as a "protein library" (Hirano 1989), containing information such as relative molecular weight, isoelectric point, amino-acid sequence, sequence homology, glycosylation and presence of disulphide bonding.

Several increasingly powerful software systems for the analysis of 2D-PAGE patterns have been developed (Anderson et al. 1985). The method using an image analyzer, in combination with molecular weight and isoelectric point marker-proteins which are commercially available, is especially useful for constructing a protein data-file. The constructed data-file should prove of use for the molecular cloning of genes. For cellular proteins that are N-terminally blocked (either naturally or artifactually during purification), the information from N-terminal deblocked and internal amino-acid sequences provides a valuable means of identification. If synthetic oligodeoxynucleotides reverse-translated from the peptide sequences compiled in the data-file are used, a gene encoding a particular protein can be efficiently and accurately cloned. Moreover, with the recent introduction of polymerase chain reaction technologies, internal sequence data greatly facilitates the cloning of cDNA encoding a protein of interest.

Rice genome projects, in which rice genes are mapped on chromosomes and cloned by "shotgun" methodology to determine nucleotide sequences, are in progress mainly in the United States and Japan. In these projects, it is required to accurately identify proteins expressed at detectable levels. 2D-PAGE in conjunction with protein micro-sequencing should serve to confirm the expression of predicted gene products.

The database provides a new approach to the analysis of gene expression in normal and transformed cells. It suggests directions for future research that might reveal relations between the functions of transforming proteins and altered patterns of protein production. It should also make possible the rapid screening of a biological sample for abnormalities in protein expression.

The rice protein library will be easily accessible to other laboratories since we are planning to input our data-file into a database to be compiled according to a world-wide standardized format.

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References

Anderson L, Anderson NG (1977) High resolution two-dimensional electrophoresis of human plasma protein. Proc Natl Acad Sci USA 74:5421–5425

Anderson NL, Nance SL, Tollaksen SL, Giere FA, Anderson NG (1985) Quantitative reproducibility of measurements from Coomassie Blue-stained two-dimensional gels: analysis of mouse liver protein patterns and a comparison of BALB/c and C57 strains. Electrophoresis 6:592–599

Bibb MJ, Van Etten RA, Wright CT, Walberg MW, Clayton DA (1981) Sequence and gene organization of mouse mitochondrial DNA. Cell 26:167–180

Chanber TM, Yamnikova S, Kawaoka Y, Lvov DK, Webster RG (1989) Antigenic and molecular characterization of subtype H13 hemaglutinin of influenza vitus. Virology 172: 180–188

Celis JE, Ratz GP, Madsen P, Gesser B, Lauridsen JB, Hansen KPB, Kwee S, Rasmussem HH, Nielsen HV, Cruger D, Basse B, Leffers H, Honore B, Moller O, Celis A (1989) Computerized, comprehensive databases of cellular and secreted proteins from normal human embryonic lung MRC-5 fibroblasts: identification of transformation and/or proliferation sensitive proteins. Electrophoresis 10:76–115

Cleveland DW, Fischer SG, Kirschner MW, Laemmli UK (1977) Peptide mapping by limited proteolysis in sodium dodecyl sulphate and analysis by gel electrophoresis. J Biol Chem 252:1102–1106

Garrels JI, Franza BR (1989) Transformation-sensitive and growth related changes of protein synthesis in REF52 cells. J Biol Chem 264:5299–5312

Hirano H (1982) Varietal differences of leaf protein profiles in mulberry. Phytochemistry 21:1513–1518

Hirano H (1989) Microsequence analysis of winged bean seed proteins electroblotted from two-dimensional gel. J Protein Chem 8:115–130

Hirano H, Watanabe T (1990) Microsequencing of proteins electrotransferred onto immobilizing matrices from polyacrylamide-gel electrophoresis: application to an insoluble protein. Electrophoresis 11:573–580

Hirano H, Komatsu S, Nakamura A, Kikuchi F, Kajiwara H, Tsunasawa S, Sakiyama F (1991) Structural homology between semidwarfism-related proteins and glutelin seed protein in rice (*Oryza sativa* L.). Theor Appl Genet 83:153–158

Jauregui-Adell J, Pechere J-F (1978) Parvalbumins from coelacanth muscle. III. Amino-acid sequence of the major component. Biochem Biophys Acta 536:275–282

Kanematsu S, Asada K (1990) Characterization of amino-acid sequences of chloroplast and cytosol isozymes of CuZn-superoxide dismutase in spinach, rice and horsetail. Plant Cell Physiol 31:99–112

Kijimoto-Ochiai S, Katagiri YU, Ochiai H (1985) Analysis of N-linked oligosaccharide chains of glycoproteins on nitrocellulose sheets using lectin-peroxidase reagents. Anal Biochem 147:222–229

Kikuchi F, Itakura N, Ikehashi H, Yokoo M, Nakane A, Maruyama K (1985) Genetic analysis of semidwarfism in high-yielding rice varieties in Japan. Bull Natl Inst Agric Sci J 36:125–145

Kondo K, Tada H, Narita K, Lee CY (1989) Amino-acid sequences of three beta-bungarotoxins (beta3-, beta4-, and beta5-bungarotoxins) from *bungarus multicictus* venom. Amino-acid substitutions in the A chains. J Biochem 91:1531–1582

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lawrence GJ, Shepherd KW (1980) Variation in glutenin protein subunits of wheat. Aust J Biol Sci 33:221–333

Matsuoka M, Kano-Murakami Y, Tanaka Y, Ozeki Y, Yamamoto N (1988) Classification and nucleotide sequence of cDNA encoding the small subunit of ribulose-1,5-bisphosphate carboxylase from rice. Plant Cell Physiol 29: 1015–1022

Nakamura A, Hirano H, Kikuchi F (1991) A protein gene *srp* (t) linked a semidwarfing gene *sd-1* in rice (*Oryza sativa* L.). Jpn J Breed 41:517–521

Rogers JC, Milliman C (1983) Isolation and sequence analysis of a barley alpha-amylase cDNA clone. J Biol Chem 258:8169–8174

Singh NK, Shepherd KW (1985) The structure and genetic control of a new class of disulphide-linked proteins in wheat endosperm. Theor Appl Genet 71:79–92

Sugiyama T, Rafalski A, Peterson D, Soll D (1985) A wheat HMW glutenin subunit gene reveals a highly repeated structure. Nucleic Acids Res 13:8729–8737

Takaiwa F, Kikuchi S, Oono K (1987) A rice glutelin family–a major type of glutelin mRNA can be divided into two classes. Mol Gen Genet 208:15–22

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

Yano H, Kamo H, Tsugita A, Aso K, Nozu Y (1989) The amino-acid sequence of plastocyanin from rice (*Oryza sativa* subspecies *japonica*). Protein Seq Data Anal 2:385–389