An Alternate cDNA Encoding Glycinin $A_{1a}B_x$ Subunit

Shigeru Utsumi,* Mitsutaka Kohno, Tomohiko Mori, and Makoto Kito

The nucleotide sequence of glycinin $A_{1a}B_x$ cDNA from *Glycine max* var. Shirotsurunoko was determined and compared with that from var. Bonminori. The comparison shows four nucleotide substitutions in the acidic polypeptide region, three of which result in amino acid replacements, three nucleotide substitutions in the basic polypeptide region, two of which are contiguous and result in an amino acid replacement, and one in the 3′-untranslated region. These differences between cultivars indicate the polymorphism of glycinin $A_{1a}B_x$ subunit gene between soybean cultivars. Three of four amino acid replacements cause changes of the side-chain properties, one of which results in dramatical change of the secondary structure around the replacement.

Glycinin, one of the predominant storage proteins of soybean (*Glycine max* L., Merr.), is composed of six subunits, each of which consists of an acidic and a basic polypeptide that are disulfide linked (Badley et al., 1975; Kitamura et al., 1976; Mori et al., 1979; Staswick et al., 1981). Recent investigations on the biosynthesis of glycinin have demonstrated that each subunit is synthesized as a single-polypeptide precursor consisting of the acidic polypeptide covalently linked to the basic polypeptide and then it is processed posttranslationally to form both acidic and basic polypeptides (Tumer et al., 1981, 1982; Barton et al., 1982). The following subunits are identified: $A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a}, A_3B_4, and $A_5A_4B_3$ (Staswick et al., 1981; Monma et al., 1985a). These subunits are classified into A_1 type ($A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a}) and A_3 type (A_3B_4, $A_5A_4B_3$) according to the similarity of NH_2-terminal amino acid sequences (Tumer et al., 1982; Nielsen, 1985).

Glycinin was regarded as a homogeneous protein with an inherent subunit composition. However, we demonstrated by means of various electrophoreses that glycinin exhibits differences in subunit compositions among the cultivars (Mori et al., 1981) and heterogeneity of the molecular species (Utsumi et al., 1981) similarly to legumins from broad bean (Utsumi and Mori, 1980, 1981; Utsumi et al., 1980) and pea (Thomson et al., 1978; Casey, 1979). Recently, Staswick et al. (1984) determined the amino acid sequence of A_2B_{1a} from var. CX635-1-1-1 and detected microheterogeneity in the sequence at 10 positions, suggesting the complexity of the gene family. More recently, the group of Fukazawa et al. prepared the nearly full-length cDNAs for $A_{1a}B_x$ (Negoro et al., 1985), A_2B_{1a} (Monma et al., 1985b), A_3B_4 (Fukazawa et al., 1985), and $A_5A_4B_3$ (Monma et al., 1985a) from var. Bonminori and determined their nucleotide sequences. The comparison of the deduced amino acid sequence of A_2B_{1a} from var. Bonminori with the amino acid sequence from var. CX635-1-1-1 (Staswick et al., 1984) shows six differences. This indicates polymorphism of a subunit molecule among soybean cultivars. From the several lines of evidence, the complexity of amino acid sequences of a glycinin subunit in a soybean cultivar and among cultivars has been proposed on the level of protein. However, the evidence on the level of gene has not been presented.

In this study we demonstrate the complete nucleotide and deduced amino acid sequences of $A_{1a}B_x$ from *G. max* var. Shirotsurunoko. The data obtained here show eight and four differences in the nucleotide and deduced amino acid sequences between var. Shirotsurunoko and Bonminori, indicating clearly the occurrence of polymorphism of glycinin $A_{1a}B_x$ subunit gene between soybean cultivars.

The Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan.

MATERIALS AND METHODS

Materials. Ribonucleoside–vanadyl complex was obtained from New England BioLabs. Oligo(dT)-cellulose, AMV reverse transcriptase, terminal deoxynucleotidyl transferase, *Escherichia coli* DNA ligase, 3′-oligo(dT)-tailed pSV7186-derived plasmid primer, and 3′-oligo-(dG)-tailed pSV1982-derived HindIII linker were from Pharmacia; M13 mp18, 19 RFI DNA, T_4DNA ligase, and restriction enzymes (AccI, AluI, EcoRI, HaeIII, HindIII, HpaII, PstI, PvuII, Sau3AI, SmaI, TaqI) were from Toyobo; RNasin and M13 sequencing kit were from Takara Shuzo; wheat germ extract, M13 cloning kit, [^{35}S]-methionine, α-^{32}P-dCTP, and α-^{32}P-ddCTP were from Amersham.

Preparation of RNA. Midmaturation-stage soybean (var. Shirotsurunoko) cotyledons were crushed in liquid N_2 by a coffee mill. Cotyledon powder (40 g) was mixed with 200 mL of extraction buffer (pH 7.8, 0.1 M Tris-HCl, 0.25 M sucrose, 2 mM dithiothreitol, 10 mM $MgCl_2$, 0.2 M NaCl, 10 mM ribonucleoside–vanadyl complex). The mixture was stirred for 45 min at 4 °C and then centrifuged for 30 min at 9000 rpm at 4 °C. The supernatant was filtered through four layers of gauze. The filtrate was mixed with $^1/_{20}$ vol of 2 M Tris, 10% SDS, and 1.1 vol of 80% phenol, and the resultant mixture was shaken for 15 min and centrifuged. The aqueous phase was extracted two times with an equal volume of phenol–chloroform–isoamyl alcohol (25:24:1) and once with chloroform. The RNA was precipitated by the addition of $^1/_{10}$ vol of 20% KOAc (pH 5.4) and 2.2 vol of ethanol and the resultant mixture stored at −20 °C for 16 h. The RNA pellet was recovered by centrifugation and washed by 70% ethanol. The pellet was dissolved in 40 mL of sterile water and then mixed with 10 mL of 10 M LiCl to eliminate DNA, tRNA, and polysaccharide (Palmiter, 1974). After the mixture was allowed to stand at 4 °C for 16 h, the precipitate was collected by centrifugation. The RNA was washed with 2 M LiCl, dissolved in water, and again precipitated by ethanol. The resultant precipitate was rinsed two times with 70% ethanol and dried in vacuo. The RNA (total RNA) was dissolved in sterile water.

Polyadenylated RNA was prepared from the total RNA by oligo(dT)-cellulose column chromatography (Aviv and Leder, 1972) and precipitated by ethanol. The polyadenylated RNA was dissolved in 10 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA, 0.5% sodium dodecyl sulfate (SDS), and 0.1 M NaCl, and the resultant mixture was heated for 10 min at 65 °C and then chilled on ice immediately. The heated polyadenylated RNA was frac-

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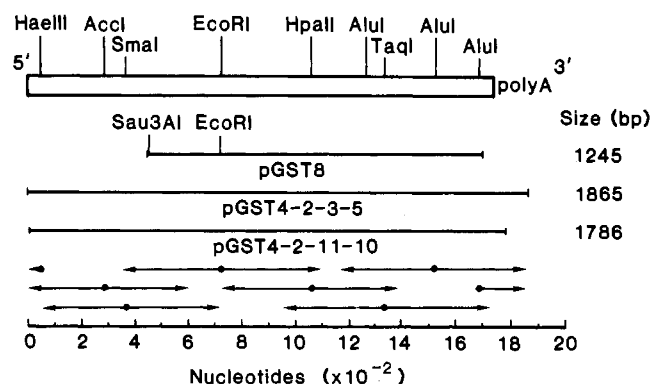

Figure 1. Size and restriction endonuclease map of $A_{1a}B_x$ cDNA clones and sequencing strategy. Since all of the cDNA clones contained the same restriction enzyme sites, the same sequencing strategy was used. The horizontal arrows show the direction and distance in which sequences were obtained. The exact nucleotide length of each cDNA clone was determined by nucleotide sequencing and is shown to the right.

tionated by centrifugation on 10–35% (w/v) linear sucrose density gradient in the above buffer at 25000 rpm and 20 °C for 17 h in a Hitachi RPS 40T rotor. After centrifugation, the gradient was divided into 0.5-mL fractions and analyzed by wheat germ cell-free translation as described below.

Cell-Free Translation and Analysis of the Products. Cell-free translation of RNA in each fraction from the sucrose density gradients was carried out with wheat germ extract and [^{35}S]methionine according to the protocol of Amersham. The optimum potassium concentration for translation of glycinin mRNA was 150–170 mM.

After the translation, the translation products were precipitated by the addition of 5 vol of cold acetone, stored at −20 °C for 16 h, centrifuged, and washed two times with 83% acetone. The pellet was dried for 30 min at room temperature, dissolved in the SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, and subjected to electrophoresis on a SDS-polyacrylamide slab gel (10%) according to the procedure of Laemmli (1970). After electrophoresis, the gel was stained with Coomasie Brilliant Blue R-250 in 10% acetic acid containing 30% methanol and destained with 7% acetic acid containing 30% methanol. The destained gel was treated with ENHANCE and fluorographed according to the protocol of New England Nuclear. The glycinin mRNA-rich fractions were collected and subjected to the preparation of a cDNA library encoding glycinin subunits.

Preparation and Identification of cDNA Encoding Glycinin Subunits. Plasmid-primed double-stranded cDNA was synthesized from the glycinin mRNA-rich fractions according to the procedure of Okayama and Berg (1982). After transformation of *E. coli* C600 by the method of Hanahan (1983), the cells were plated on ampicilin-containing agar plates and replicated on nitrocellulose filters. A total of 2000 transformants was screened by colony hybridization (Maniatis et al., 1982). Prior to this study, we succeeded in preparing a cDNA (pGST8, 1245bp) arising from the center of the acidic polypeptide to the 3′-untranslated region of an A_1-type subunit (see the text) employing the method of Norgard et al. (1980) (see Figure 1). An approximately 250-bp fragment derived from the 5′-end region of pGST8 was cleaved by EcoRI and Sau3AI, 3′-end labeled with terminal deoxynucleotidyl transferase and ^{32}P-ddCTP (Yousaf et al., 1984), and used as a probe. Plasmids from the positive clones were isolated by the boiling method (Maniatis et al., 1982), and the sizes were analyzed by electrophoresis in 0.7% agarose gels. Plasmids in which the sizes of the inserts were longer than 1800 bp were analyzed by several restriction enzymes. Then we selected two clones (pGST4-2-3-5, pGST4-2-11-10) as possible candidates encoding $A_{1a}B_x$. These two plasmids were purified as described by Maniatis et al. (1982) and subjected to nucleotide sequence analysis.

Nucleotide Sequence Analysis. The inserts of pGST4-2-3-5 and pGST4-2-11-10 were cleaved by some restriction enzymes (Figure 1). The digested fragments were subcloned into M13 mp18 and 19, and single-stranded templates were prepared according to the protocol of Amersham. Nucleotide sequence was determined by the dideoxy-sequencing method (Sanger et al., 1977) employing the sequencing kit of Takara Shuzo.

Computer Analysis of the DNA Sequence. The secondary structure was predicted according to the procedure of Chou and Fasman (1974a, 1974b). Search distances of 6 and 4 were used for helical and sheet structures, respectively. For the β-turn analysis, tetrapeptides with $P_t > 0.75 \times 10^{-4}$ were selected as probable turns.

RESULTS

Isolation of the cDNA Encoding $A_{1a}B_x$ Subunit. Prior to this study, we prepared a soybean cDNA library according to the classical procedure employing S_1-nuclease and selected pGST8, with a cDNA insert of 1245 bp (see Figure 1), encoding a glycinin subunit by means of positive hybridization selection. The nucleotide sequence of the insert of pGST8 was determined and compared with NH_2-terminal amino acid sequences of glycinin subunits reported by Tumer et al. (1982) and Nielsen (1985) and nucleotide sequences of glycinin subunit cDNAs determined by the group of Fukazawa et al. (Monma et al., 1985a, 1985b; Negoro et al., 1985; Fukazawa et al., 1985). This indicates that pGST8 encodes the $A_{1a}B_x$ subunit. In the present study, a glycinin mRNA-rich fraction obtained by sucrose density gradient centrifugation was used as template for preparation of a cDNA library according to the procedure of Okayama and Berg (1982) (see Materials and Methods). About 2000 clones were screened with the ^{32}P-3′-end-labeled Sau3AI-EcoRI fragment (approximately 250 bp; see Figure 1) of pGST8, and 10 clones were selected as possible candidates containing A_1-type cDNAs, the sizes of which are longer than 1800 bp.

The cDNA inserts were cleaved by several restriction enzymes, and the restriction endonuclease map was illustrated as shown in Figure 1. Comparing the map with that of $A_{1a}B_x$ from var. Bonminori reported by Negoro et al. (1985), we selected two clones (pGST4-2-3-5, pGST4-2-11-10) that seem to contain $A_{1a}B_x$ cDNA. The length of the cDNA inserts of pGST4-2-3-5 and pGST4-2-11-10 was 1865 and 1786 bp (Figure 1).

Nucleotide Sequence of $A_{1a}B_x$ cDNA from Var. Shirotsurunoko and Comparison with That from Var. Bonminori. The sequencing strategy was shown in Figure 1. The nucleotide sequence and the deduced amino acid sequence of the inserts of pGST4-2-3-5 and pGST4-2-11-10 were shown in Figure 2. Both inserts of pGST4-2-3-5 and pGST4-2-11-10 contained the entire coding region of mRNA and adjacent portions of its untranslated sequences. The sequences of both inserts are completely identical with each other, though the length of poly(A) of pGST4-2-3-5 is 125 bases and that of pGST4-2-11-10 is 50 bases. The deduced amino acid sequence corresponds completely to the NH_2-terminal sequence of A_{1a} and B_{1b} (Tumer et al., 1982; Nielsen, 1985).

Comparison of the nucleotide sequence of $A_{1a}B_x$ from var. Shirotsurunoko with that from var. Bonminori (Negoro et al., 1985) shows that four nucleotide substitutions

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                                                ACAACTCAAACATTCTCTCCATTGGTC   27

CTTAAACACTCATCAGTCATCACCATGGCCAAGCTAGTTTTTTCCCTTTGTTTTCTGCTTTTCAGTGGCTGCTGCTTCGCT  108
                        MetAlaLysLeuValPheSerLeuCysPheLeuLeuPheSerGlyCysCysPheAla   -1

TTCAGTTCCAGAGAGCAGCCTCAGCAAAACGAGTGCCAGATCCAAAAACTCAATGCCCTCAAACCGGATAACCGTATAGAG  189
PheSerSerArgGluGlnProGlnGlnAsnGluCysGlnIleGlnLysLeuAsnAlaLeuLysProAspAsnArgIleGlu   27

TCAGAAGGAGGGCTCATTGAGACATGGAACCCTAACAACAAGCCATTCCAGTGTGCCGGTGTTGCCCTCTCTCGCTGCACC  270
SerGluGlyGlyLeuIleGluThrTrpAsnProAsnAsnLysProPheGlnCysAlaGlyValAlaLeuSerArgCysThr   54

CTCAACCGCAACGCCCTTCGTAGACCTTCCTACACCAACGGTCCCCAGGAAATCTACATCCAACAAGGTAAGGGTATTTTT  351
LeuAsnArgAsnAlaLeuArgArgProSerTyrThrAsnGlyProGlnGluIleTyrIleGlnGlnGlyLysGlyIlePhe   81

GGCATGATATACCCGGGTTGTCCTAGCACATTTGAAGAGCCTCAACAACCTCAACAAAGAGGACAAAGCAGCAGACCACAA  432
GlyMetIleTyrProGlyCysProSerThrPheGluGluProGlnGlnProGlnGlnArgGlyGlnSerSerArgProGln  108

GACCGTCACCAGAAGATCTATAACTTCAGAGAGGGTGATTTGATCGCAGTGCCTACTGGTGTTGCATGGTGGATGTACAAC  513
AspArgHisGlnLysIleTyrAsnPheArgGluGlyAspLeuIleAlaValProThrGlyValAlaTrpTrpMetTyrAsn  135

AATGAAGACACTCCTGTTGTTGCCGTTTCTATTATTGACACCAACAGCTTGGAGAACCAGCTCGACCAGATGCCTAGGAGA  594
AsnGluAspThrProValValAlaValSerIleIleAspThrAsnSerLeuGluAsnGlnLeuAspGlnMetProArgArg  162

TTCTATCTTGCTGGGAACCAAGAGCAAGAGTTTCTAAAATATCAGCAAGAGCAAGGAGGTCATCAAAGCCAGAAAGGAAAG  675
PheTyrLeuAlaGlyAsnGlnGluGlnGluPheLeuLysTyrGlnGlnGluGlnGlyGlyHisGlnSerGlnLysGlyLys  189

CATCAGCAAGAAGAAGAAAACGAAGGAGGCAGCATATTGAGTGGCTTCACCCTGGAATTCTTGGAACATGCATTCAGCGTG  756
HisGlnGlnGluGluGluAsnGluGlyGlySerIleLeuSerGlyPheThrLeuGluPheLeuGluHisAlaPheSerVal  216

GACAAGCAGATAGCGAAAAACCTACAAGGAGAGAACGAAGGGGAAGACAAGGGAGCCATTGTGACAGTGAAAGGAGGTCTG  837
AspLysGlnIleAlaLysAsnLeuGlnGlyGluAsnGluGlyGluAspLysGlyAlaIleValThrValLysGlyGlyLeu  243

AGCGTGATAAAACCACCCACGGACGAGCAGCAACAAAGACCCCAGGAAGAGGAAGAAGAAGAAGAGGATGAGAAGCCACAG  918
SerValIleLysProProThrAspGluGlnGlnGlnArgProGlnGluGluGluGluGluGluGluAspGluLysProGln  270

TGCAAGGGTAAAGACAAACACTGCCAACGCCCCCGAGGAAGCCAAAGCAAAAGCAGAAGAAATGGCATTGACGAGACCATA  999
CysLysGlyLysAspLysHisCysGlnArgProArgGlySerGlnSerLysSerArgArgAsnGlyIleAspGluThrIle  297

TGCACCATGAGACTTCGCCACAACATTGGCCAGACTTCATCACCTGACATCTACAACCCTCAAGCCGGTAGCGTCACAACC 1080
CysThrMetArgLeuArgHisAsnIleGlyGlnThrSerSerProAspIleTyrAsnProGlnAlaGlySerValThrThr  324

GCCACCAGCCTTGACTTCCCAGCCCTCTCGTGGCTCAGACTCAGTGCTGAGTTTGGATCTCTCCGCAAGAATGCAATGTTC 1161
AlaThrSerLeuAspPheProAlaLeuSerTrpLeuArgLeuSerAlaGluPheGlySerLeuArgLysAsnAlaMetPhe  351

GTGCCACACTACAACCTGAACGCGAACAGCATAATATACGCATTGAATGGACGGGCATTGATACAAGTGGTGAATTGCAAC 1242
ValProHisTyrAsnLeuAsnAlaAsnSerIleIleTyrAlaLeuAsnGlyArgAlaLeuIleGlnValValAsnCysAsn  378

GGTGAGAGAGTGTTTGATGGAGAGCTGCAAGAGGGACGGGTGCTGATCGTGCCACAAAACTTTGTGGTGGCTGCAAGATCA 1323
GlyGluArgValPheAspGlyGluLeuGlnGluGlyArgValLeuIleValProGlnAsnPheValValAlaAlaArgSer  405

CAGAGTGACAACTTCGAGTATGTGTCATTCAAGACCAATGATACACCCATGATCGGCACTCTTGCAGGGGCAAACTCATTG 1404
GlnSerAspAsnPheGluTyrValSerPheLysThrAsnAspThrProMetIleGlyThrLeuAlaGlyAlaAsnSerLeu  432

TTGAACGCATTACCAGAGGAAGTGATTCAGCACACTTTCAACCTAAAAAGCCAGCAGGCCAGGCAGATAAAGAACAACAAC 1485
LeuAsnAlaLeuProGluGluValIleGlnHisThrPheAsnLeuLysSerGlnGlnAlaArgGlnIleLysAsnAsnAsn  459

CCTTTCAAGTTCCTGGTTCCACCTCAGGAGTCTCAGAAGAGAGCTGTGGCTTAGAGCCCTTTTTGTATGTGCTACCCCACT 1566
ProPheLysPheLeuValProProGlnGluSerGlnLysArgAlaValAla                               476

TTTGTCTTTTTGGCAATAGTGCTAGCAACCAATAAATAATAATAATAATAATGAATAAGAAAACAAAGGCTTTAGCTTGCC 1647

TTTTGTTCACTGTAAAATAATAATGTAAGTACTCTCTATAATGAGTCACGAAACTTTTGCGGGAATAAAGGAGAAATTCC 1728

AATGAGTTTTCTGTC-poly(A)                                                          1743
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Figure 2. Nucleotide and deduced amino acid sequences of $A_{1a}B_x$ cDNA from var. Shirotsurunoko. Amino acid residues are numbered in the NH_2 to COOH direction starting with the NH_2-terminal residue of the mature acidic polypeptide. * and ** indicate the NH_2-terminal residues of the mature acidic and basic polypeptides, respectively. The AATAAA sequences in the 3′-untranslated region are underlined. O indicates the nonidentical nucleotides between var. Shirotsurunoko and Bonminori.

Table I. Differences in the Nucleotide and Amino Acid Sequences $A_{1a}B_x$ cDNA between Var. Shirotsurunoko and Bonminori

	nucleotide			amino acid		
region	no.	ST[a]	BM[a]	no.	ST	BM
acidic polypeptide	176	A	G	23	Asp	Gly
	318	G	A	70	Gln	Gln
	373	C	T	89	Pro	Ser
	458	T	C	117	Phe	Ser
basic polypeptide	1130	A	G	341	Glu	Gly
	1131	G	A			
	1137	A	G	343	Gly	Gly
3′-untranslated	1740	C	T			

[a] ST and BM refer to Shirotsurunoko and Bonminori, respectively.

Table II. Effects of Amino Acid Replacements on Side-Chain Properties of the Residues and the Secondary Structure around the Amino Acid Replacements

residue no.	amino acids		hydropathy index[b]		secondary structure	
	ST[a]	BM[a]	ST	BM	ST	BM
23	Asp	Gly	−3.5	−0.4	β turn	β turn
89	Pro	Ser	−1.6	−0.8	β turn	β turn
117	Phe	Ser	2.8	−0.8	random	random
341	Glu	Gly	−3.5	−0.4	α helix	β turn

[a] ST and BM refer to Shirotsurunoko and Bonminori, respectively. [b] The index from Kyte and Doolittle (1982).

occur in the acidic polypeptide region, three of which result in amino acid replacements, and that three nucleotide substitutions are seen in the basic polypeptide region, two of which are contiguous and result in one amino acid replacement (Table I). In addition, there is one nucleotide substitution in the 3′-untranslated region (Table I). It is noted that nucleotide substitutions occur between A and G and between C and T.

Effects of Amino Acid Replacements on Side-Chain Properties and Secondary Structure. Four differences in the deduced amino acid sequences of $A_{1a}B_x$ were observed between var. Shirotsurunoko and var. Bonminori. Such differences may change the side-chain properties of

amino acids at these positions, altering for example the hydropathy index (Kyte and Doolittle, 1982) as shown in Table II. It is reasonable to expect that such changes except at the 89th residue may affect the tertiary structure of the subunit. The secondary structure of the $A_{1a}B_x$ subunit from both cultivars was predicted according to the procedure of Chou and Fasman (1974a, 1974b). The secondary structure around the amino acid replacement was summarized in Table II. It was predicted that the 23rd, the 89th, and the 117th residues cause no change but the 341st residue may cause a change.

DISCUSSION

Investigations on the complexity of the amino acid sequence of a glycinin subunit in a soybean cultivar and among soybean cultivars have progressed on the level of the primary structure. However, sufficient information on the level of the gene is lacking at present. Recently, the nucleotide sequences of a partial cDNA and a partial genomic DNA of the A_2B_{1a} subunit and partial cDNAs of A_3B_4 and $A_5A_4B_3$ subunits from var. CX635-1-1-1 have been proposed by the group of Nielsen et al. (Marco et al., 1984; Scallon et al., 1985). In addition, the nucleotide sequences of the nearly full-length cDNAs for $A_{1a}B_x$, A_2B_{1a}, A_3B_4, and $A_5A_4B_3$ subunits from var. Bonminori have been determined by the group of Fukazawa et al. (Monma et al., 1985a, 1985b; Negoro et al., 1985; Fukazawa et al., 1985). With regard to the A_2B_{1a} subunit there are two differences in the nucleotide sequences between cDNAs from var. CX635-1-1-1 and Bonminori and between cDNA from var. Bonminori and genomic DNA from var. CX635-1-1-1 but no replacement in the deduced amino acid sequences. In the case of the A_3B_4 subunit, there are 35 changes out of the 560 bases as well as some deletions and insertions. In the case of the $A_5A_4B_3$ subunit, nine differences out of the 664 bases and three deletions (insertions) are observed. Further, the nucleotide sequences of A_3B_4 and $A_5A_4B_3$ reported by Scallon et al. (1985) are about one-third of the full size. On the other hand, Hirano et al. (1984, 1985) presented the complete amino acid sequences of A_3 and A_4 polypeptides from var. Bonminori. The results disagree with those from the groups of Nielsen and Fukazawa et al. Therefore, it is difficult to say whether they are working on the same subunits. These available data cannot propose the idea that polymorphism occurs at the level of gene.

The nucleotide and deduced amino acid sequences of the $A_{1a}B_x$ subunit from var. Shirotsurunoko were compared here with those from var. Bonminori (Negoro et al., 1985) (Table I). Eight differences in the nucleotide sequence are observed. Seven of these differences are in the coding DNA, five of which result in amino acid replacements at four positions. The fact suggests either that the gene encoding the $A_{1a}B_x$ subunit is different between cultivars or that the cDNA studied is not encoding $A_{1a}B_x$ but $A_{1b}B_y$. However, the COOH-terminal amino acid sequences of A_{1a} and A_{1b} described in a review by Nielsen (1985) are completely different. The results by Negoro et al. (1985) and by us indicate that the deduced amino acid sequences correspond to A_{1a}. This supports the conclusion that there is polymorphism of $A_{1a}B_x$ subunit genes.

Staswick et al. (1981) reported that the counterpart of A_{1a} is B_2 in var. CX635-1-1-1. Therefore, Negoro et al. (1985) adopted the designation of $A_{1a}B_x$. However, Nielsen (1985) described in the latest review that the counterpart of A_{1a} seems to be B_{1b} in var. Dare. The results obtained here and by Negoro et al. (1985) suggest a possible combination of A_{1a} and B_{1b}. There are two possibilities: one, the counterpart of A_{1a} is different in different cultivars; two, the combination of $A_{1a}B_2$ does not occur.

Negoro et al. (1985) stated that they determined the nucleotide sequences of 14 $A_{1a}B_x$ cDNAs and observed no nucleotide substitutions among them. This suggests no microheterogeneity in the A_{1a} subunit precursor protein. However, Staswick et al. (1984) observed microheterogeneity at 10 positions in the amino acid sequence of A_2B_{1a} subunit from var. CX635-1-1-1, suggesting that the polymorphism of the A_2B_{1a} gene is fixed. We observed that pGST8 has three differences in the nucleotide and deduced amino acid sequences with pGST4-2-3-5 and pGST4-2-11-10 (data not shown). This indicates the polymorphism of the gene encoding the $A_{1a}B_x$ subunit occurring in var. Shirotsurunoko and supports the above assumption. However, it is necessary to determine the amino acid sequence of the $A_{1a}B_x$ subunit from var. Shirotsurunoko to confirm the polymorphism. It does not exclude the possibilities that the cDNAs obtained here are derived from pseudogenes at the level of protein stability and that a part of microheterogeneity results from posttranslational modification.

Polymorphism of the gene may be caused by mutation during duplication and crossing of a common ancestral gene. It is reasonable to expect that a gene that suffered a mutation and cannot construct a given glycinin structure has not been conserved. Argos et al. (1985) demonstrated that the basic polypeptide regions are highly conservative among glycinin subunits. However, we have observed an amino acid replacement at the 341st position, which causes the difference in the secondary structure predicted according to the procedure of Chou and Fasman (1974a, 1974b) (Table II). This suggests that even highly conservative region may allow a partial conformational change. Therefore, it is useful to determine the nucleotide sequences of allelic variants of glycinin subunits in identifying sites of permissive changes in protein primary structure and for development of genetic engineering of seed proteins.

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Registry No. DNA (soybean clone pGST4-2-3-5 glycinin $A_{1a}B_x$ messenger RNA complementary), 106434-22-4; glycinin (soybean clone pGST4-2-3-5 $A_{1a}B_x$ precursor reduced), 106434-23-5; glycinin (soybean clone pGST4-2-3-5 A_{1a} subunit reduced), 106434-24-6; glycinin (soybean clone pGST4-2-3-5 B_x subunit reduced), 106434-25-7.

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Hemicellulose Monosaccharide Composition and in Vitro Disappearance of Orchard Grass and Alfalfa Hay

Cindy L. Wedig, Edwin H. Jaster,* and Kenneth J. Moore

Samples of alfalfa and orchard grass hays were incubated in rumen fluid for 0, 6, 12, 18, 24, 36, 48, and 72 h to compare monosaccharide composition and extent and rate of disappearance of the two forages. Hemicellulose monosaccharides were quantified in the neutral detergent fiber (NDF) residue of each sample. The xylose concentration of alfalfa increased more rapidly during in vitro fermentation than did that of orchard grass. Xylose to arabinose ratios increased with digestion time for both alfalfa and orchard grass, suggesting that the arabinose component of hemicellulose is more digestible than is the xylose component. Xylose to glucose ratios increased for both alfalfa and orchard grass, suggesting that the cellulose component is more rapidly degraded than is hemicellulose. The extent of NDF digestion was higher for orchard grass than for alfalfa. Uronic acids and glucose were the cell wall monosaccharides that were more digestible in orchard grass than in alfalfa.

The majority of feed and food energy in the world is in the form of plant material, which is not utilizable by humans. The study of plant cell wall components is important, not only for a basic understanding of structural integrity but also for accurate determination of nutrient availability to ruminants. Cellulose, hemicellulose, and pectin are the major structural polysaccharides found in plant material. Hemicellulose is the most complex of the fiber components of common forages (Bailey, 1973; Van Soest, 1982; Wilkie, 1979), and it needs better definition and characterization.

On grass diets, both ruminants and nonruminants digest more hemicellulose than cellulose (Daughtry et al., 1978; Keys et al., 1967; Van Soest, 1982). On legume diets, ruminants digest more cellulose than hemicellulose, but nonruminants still digest more hemicellulose than cellulose (Keys et al., 1967). Grasses show a wider range in hemicellulose digestibility values than does alfalfa (Van Soest, 1973). An increase in total structural polysaccharides fed to humans has been shown by Slavin and co-workers (1983) to decrease dry-matter digestibility of the diet. In a digestibility trial with sheep, Sullivan (1966) demonstrated that dry-matter (DM) digestibility and percent hemicellulose were significantly negatively correlated.

Departments of Animal Sciences (C.L.W., E.H.J.) and Agronomy (K.J.M.), University of Illinois, Urbana, Illinois 61801.

Ruminants have the capability to digest all structural polysaccharides. The majority of hemicellulose digestion takes place in the rumen and abomasum (Dehority, 1973). Mean rumen turnover time for fiber particles is 30-50 h (Van Soest, 1973). Since grasses usually take longer to completely break down than do legumes, their digestibilities tend to be lower than digestibilities of alfalfa (Van Soest, 1973).

The digestion of hemicellulose is subject to interference by lignin (Bailey, 1973; Bittner, 1983; Burdick and Sullivan, 1963; Van Soest, 1973, 1982). The digestibility of hemicellulose is closely related to that of cellulose and negatively related to lignification. Sullivan (1966) calculated a correlation coefficient of −0.83 between hemicellulose digestibility and lignin content in alfalfa.

Hemicellulose forms bonds with lignin (Van Soest, 1982; Wilkie, 1979). Use of ^{13}C nuclear magnetic resonance (NMR) indicates the presence of lignin-carbohydrate complexes (Barton et al., 1982). These lignin-carbohydrate complexes result in decreased recoveries when acid hydrolysis is used to extract hemicellulose (Barton et al.,

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