Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 6745–6749, October 1987
Botany

Isolation and characterization of a cDNA clone encoding wheat germ agglutinin

(lectin/wheat)

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Communicated by Anton Lang, June 8, 1987 (received for review April 20, 1987)

ABSTRACT **Two sets of synthetic oligonucleotides coding for amino acids in the amino- and carboxyl-terminal portions of wheat germ agglutinin were synthesized and used as hybridization probes to screen cDNA libraries derived from developing embryos of tetraploid wheat. The nucleotide sequence for a cDNA clone recovered from the cDNA library was determined by dideoxynucleotide chain-termination sequencing in vector M13. The amino acid sequence deduced from the DNA sequence indicated that this cDNA clone (pNVR1) encodes isolectin 3 of wheat germ agglutinin. Comparison of the deduced amino acid sequence of clone pNVR1 with published sequences indicates isolectin 3 differs from isolectins 1 and 2 by 10 and 8 amino acid changes, respectively. In addition, the protein encoded by pNVR1 extends 15 amino acids beyond the carboxyl terminus of the published amino acid sequence for isolectins 1 and 2 and includes a potential site for N-linked glycosylation. Utilizing the insert of pNVR1 as a hybridization probe, we have demonstrated that the expression of genes for wheat germ agglutinin is modulated by exogenous abscisic acid. Striking homology is observed between wheat germ agglutinin and chitinase, both of which are proteins that bind chitin.**

Lectins, sugar-binding proteins derived mainly from plant sources, have been of great value as specific probes for investigating the distribution and function of carbohydrates on the surfaces of animal cells (1, 2). In recent years, however, the notion has become widely accepted that the ability of lectins to distinguish discrete sugars did not arise fortuitously during evolution (2), and as a result, there has been increased interest in the synthesis and biochemistry of this group of proteins. Wheat germ agglutinin (WGA), the first cereal lectin characterized in detail, binds specifically to the sugar *N*-acetylglucosamine and to chitin, a polymer of *N*-acetylglucosamine residues (3, 4). In the hexaploid wheat *Triticum aestivum*, WGA exists as three closely related isolectins derived from the A, B, and D genomes (5, 6). Comparison of the amino acid sequences for isolectin 1 (A genome) and isolectin 2 (D genome) indicates that these proteins differ at four residues (7, 8). The amino acid sequence for isolectin 3 (B genome), the least abundant form, is not yet available. These three isolectins randomly associate into functional dimers *in vivo* (5) and are immunologically indistinguishable (9).

In wheat plants, WGA is found in the embryos and adventitious roots (9–11). During embryogenesis, WGA expression is under temporal control (12). Accumulation of WGA is tissue-specific and cell-type specific in various organs of the embryo (e.g., coleoptile, coleorhiza, and radicle) (9, 10). In other species of Gramineae, a lectin immunologically related to WGA is synthesized during seed development and in the roots of adult plants (13, 14). Furthermore, the accumulation of lectin is modulated by the hormone abscisic acid (12, 15). Although biochemical, immunological, and microscopic studies have helped to characterize the composition and distribution of WGA (3–8, 10, 11), the genes for WGA have not been isolated.

We are interested in investigating the molecular mechanisms that regulate the developmental tissue-specific expression of WGA genes. To isolate clones for WGA, cDNA libraries from developing grains of the tetraploid wheat *Triticum durum* (AABB) were used. Here, we report the isolation and the nucleotide sequence of a cDNA clone that we presume to encode isolectin 3.* Using this clone as a hybridization probe, we present evidence that the expression of WGA genes is modulated by abscisic acid. Because of the common ability of WGA and chitinase to bind chitin, we searched for amino acid homology using the recently published sequence for chitinase from *Phaseolus vulgaris* (16). We found strong homology between the amino terminus of chitinase and four regions of WGA. The significance of this similarity is addressed.

MATERIALS AND METHODS

Plant Material. Wheat *T. aestivum* L. (AABBDD) cv. Marshall was obtained from the Minnesota Crop Improvement Association (St. Paul, MN). Plants were grown as previously described (11), and embryos were collected at 20 days after bloom (anthesis) according to Raikhel and Quatrano (12). Abscisic acid treatment involved culturing isolated embryos in the dark at 27°C for 3 days on filter paper containing growth medium (15) with and without 10^{-4} M abscisic acid (Sigma).

Materials. Two cDNA libraries, derived from mRNA isolated from developing wheat grains of *T. durum* (AABB) cv. Mexicali at 3 and 4 weeks post-anthesis, were provided by C. Brinegar (ARCO Plant Cell Research Institute, Dublin, CA). Two sets of degenerate synthetic oligonucleotides were prepared for amino acid regions in isolectin 1 (8): for the sequence Asn-Met-Glu-Cys-Pro-Asn-Asn in the amino-terminal region (residues 9–15), probe 2, TTR TAC CTY ACR GGN TTR TT; and for the sequence Cys-Thr-Asn-Asn-Tyr-Cys-Cys in the carboxyl terminal region (residues 141–147), probe 1, ACR TGN TTR TTR ATR ACR AC. The oligonucleotide mixtures were synthesized on an Applied Biosystems (Foster City, CA) 380 DNA synthesizer by a solid-phase method (17) and separated by electrophoresis on a 20% polyacrylamide gel containing 8 M urea in TBE, pH 8.3 (0.89 M Tris/0.089 M boric acid/2.7 mM EDTA). The oligonucleotides were eluted in 0.5 M ammonium acetate/10 mM magnesium acetate/0.1% $NaDodSO_4$, and then end-labeled with ^{32}P using T4 polynucleotide kinase (18).

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Abbreviation: WGA, wheat germ agglutinin.

*This sequence of isolectin 3 of wheat germ agglutinin is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J02961).

Isolation and Screening of cDNA Clones. The cDNA libraries, in *Escherichia coli* strain DH5α (19), were plated directly onto nitrocellulose filters laid on agar plates containing Luria broth medium with ampicillin at 50 μg/ml (20). After colonies were established, the bacteria were lysed, and the filters were probed with oligonucleotide probes 1 and 2 as follows. The temperature of hybridization (T_H) for each oligonucleotide was calculated using the formula $T_H = T_D - 12°C$, where T_D = 2°C × (the number of A·T base pairs) plus 4°C × (the number of G·C base pairs). Replicate filters were prehybridized in 6× SSC (0.9 M sodium chloride and 0.09 M sodium citrate, pH 7.0) (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7) plus 0.25% nonfat milk, and hybridized in the same buffer containing the labeled oligonucleotide probes and 0.1% $NaDodSO_4$ at 36°C (probe 1) or 38°C (probe 2). After hybridization, filters were washed three times in 6× SSC/0.25% nonfat milk/0.1% $NaDodSO_4$ at room temperature for 10 min, followed by a 2-min wash at 46°C (probe 1) or at 48°C (probe 2). Filters were dried and autoradiographed for 16–18 hr. Colonies that produced positive signals were selected and rescreened using the same probes under the same conditions.

DNA Sequence Determination. Inserts from recombinant plasmids were purified by electrophoresis in low-melting-point agarose. Excised cDNA inserts or appropriate restriction fragments were then subcloned into M13mp18 or M13mp19. Dideoxynucleotide chain-termination sequencing from single-stranded M13 templates was accomplished using a Bethesda Research Laboratories M13 sequencing kit with the exception that dGTP was replaced by 7-deaza-2′-deoxyguanosine triphosphate (Boehringer Mannheim).

RNA Blot Analysis. Total RNA was isolated as described (21) and poly(A)$^+$ RNA was purified by chromatography on oligo(dT)-cellulose (18). Poly(A)$^+$ RNA was electrophoresed in adjacent lanes (1 μg per lane) on 2% agarose gels containing 6% formaldehyde and then transferred to nitrocellulose (22). Filters were hybridized with inserts labeled by the random primer method of Feinberg and Vogelstein (23) and washed under stringent conditions as described in Thomas (22).

RESULTS

Isolation of cDNA Clones. Two synthetic oligonucleotides, each consisting of 20 nucleotides complementary to the 5′ and 3′ ends of the coding portion of isolectin 1 mRNA (8), were used for isolation of cDNA clones specific for WGA. These two sequences corresponded to amino acids 9–15 (probe 2) and 141–147 (probe 1). Because of the degeneracy of the sequences involved, probe 2 was a mixture of 64 sequences, and probe 1 was a mixture of 128 sequences. One cDNA clone, pNVR1 [1.0 kilobase (kb)], was selected by hybridization to both probes on the assumption that this insert contains sequences spanning the coding region delimited by the oligonucleotide probes. A second clone, pNVR2 (0.7 kb), was recognized by probe 1 only and is presumably truncated at the 5′ end. The restriction map and partial sequence of pNVR2 indicate that it is a shorter version of pNVR1. When the insert from clone pNVR1 was labeled by the random primer method (23) and used as probe to rescreen the cDNA libraries, no additional cDNA clones were retrieved.

Nucleotide Sequence. The cDNA insert of pNVR1 was subcloned into M13mp18 and M13mp19 according to the strategy shown in Fig. 1, and its nucleotide sequence was determined as described. The nucleotide sequence of the cDNA clone and the deduced amino acid sequence are shown in Fig. 2. Clone pNVR1 contains a 558-nucleotide open reading frame encoding a 186-amino acid polypeptide rich in cysteine and glycine but lacking an ATG start codon at the 5′ end. Protein sequence analysis indicates that the amino terminus of WGA is blocked (7, 8) so that the first residue (glutamine) of the published sequence may not be the amino terminus of mature WGA. It is, therefore, presumably fortuitous that the cDNA clone pNVR1 and the published amino acid sequence for WGA initiate with the same amino acid. The hydropathy plot (24) of the polypeptide encoded by clone pNVR1 shows the polypeptide to be comprised mostly of hydrophilic amino acids (Fig. 3). The polypeptide encoded by pNVR1 extends 15 amino acids beyond the carboxyl terminus of the amino acid sequence published for isolectins 1 and 2 (Fig. 2, squares) (7, 8). The carboxyl-terminal segment contains the most hydrophobic portion of the entire protein (Fig. 3). A potential site for N-linked glycosylation occurs at residues 180–182 (Asn-Ser-Thr) (Fig. 2, dots above squares). The 3′-untranslated region contains four in-frame termination codons (TGA, TGA, TAA, and TAG, underlined in Fig. 2) and a potential polyadenylylation signal (AATAAT, double-underlined in Fig. 2), and terminates in a poly(A) tail that begins 229 nucleotides downstream from that signal.

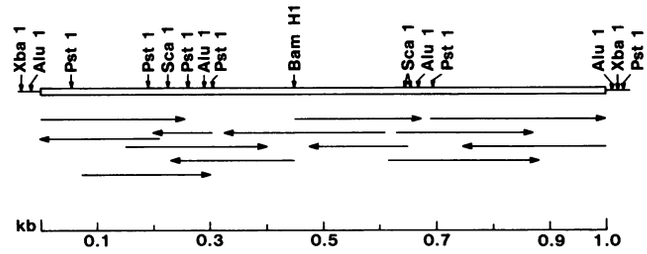

FIG. 1. Restriction map and sequencing strategy for WGA cDNA clone pNVR1. Open bar, cloned cDNA; arrows, length and direction of the sequenced restriction fragments. Scale of the map is in kb pairs.

Comparison with Published Sequences of Isolectin 1 and 2. The amino acid sequence deduced from the cDNA nucleotide sequence (Fig. 2) was compared with published protein sequence data. Re-evaluation of the discrepancies at positions 134 and 150 (Fig. 2, arrows) has indicated a low yield of lysine in addition to glycine for residue 134 (C. Wright, personal communication) and has confirmed the presence of tryptophan at residue 150 (25). The deduced amino acid sequence of pNVR1 was found to differ from the published sequence of isolectin 1 (8) at 10 positions and isolectin 2 at 8 positions (7) (Table 1).

RNA Blot Analysis. Embryos isolated from hexaploid wheat at 20 days post-anthesis were cultured in the presence and absence of abscisic acid (Fig. 4). Equal amounts of poly(A)$^+$ RNA from the embryos were fractionated by agarose-formaldehyde gel electrophoresis and transferred to nitrocellulose filters. A 1.1-kb mRNA was detected (Fig. 4) after hybridization with pNVR1 insert labeled by the random primer method (23). The autoradiograph showed that the level of RNA in abscisic acid-treated embryos was several times higher than the level in embryos cultured in the absence of abscisic acid.

Nucleotide and Amino Acid Homology Between WGA and Chitinase. The deduced amino acid sequence of cDNA clone pNVR1 was used to search for homology with chitinase, an enzyme that catalyzes the hydrolysis of 1,4-β linkages of *N*-acetylglucosamine polymers in chitin. The amino acid homology matrix between clone pNVR1 and chitinase from *P. vulgaris* is shown in Fig. 5. This matrix was generated using the analysis program of Pustell and Kafatos (26) with parameters set so that each letter within the matrix represents a match of 50% or greater over a span of 21 amino acids. Extensive homology between the amino terminus of chitinase and four regions of WGA is apparent.

DISCUSSION

In this paper we present the amino acid sequence of WGA as deduced from a cDNA clone designated pNVR1. That this clone encodes WGA has been verified by comparison of the

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                                  Asn                                       20
gln arg cys gly glu gln gly ser gly met glu cys pro asn asn leu cys cys ser gln tyr gly tyr cys gly met

CAA AGG TGC GGC GAG CAG GGC AGC GGC ATG GAG TGC CCC AAC AAC CTG TGC TGC AGC CAG TAC GGC TAC TGC GGG ATG

                                        Asp         40
gly gly asp tyr cys gly lys gly cys gln asn gly ala cys trp thr ser lys arg cys gly ser gln ala gly gly

GGC GGC GAT TAC TGC GGC AAG GGC TGC CAG AAC GGC GCG TGC TGG ACC AGC AAG CGG TGT GGC AGC CAG GCC GGC GGC

Ala          *          * 60                    *
lys thr cys pro asn asn his cys cys ser gln tyr gly his cys gly phe gly ala glu tyr cys gly ala gly cys

AAG ACG TGC CCC AAC AAC CAC TGC TGC AGC CAG TAC GGG CAC TGC GGC TTC GGC GCG GAG TAC TGC GGC GCC GGC TGC

    80                                              Ser                       100
gln gly gly pro cys arg ala asp ile lys cys gly ser gln ala gly gly lys leu cys pro asn asn leu cys cys

CAG GGC GGC CCC TGC CGC GCC GAC ATC AAG TGC GGC AGC CAG GCC GGC GGC AAG CTG TGC CCC AAC AAC CTC TGC TGC

                Ser                                     Gly 120         Ser
ser gln trp gly tyr cys gly leu gly ser glu phe cys gly glu gly cys gln asn gly ala cys ser thr asp lys

AGC CAG TGG GGG TAC TGC GGC CTC GGT TCC GAG TTC TGC GGC GAG GGC TGC CAG AAC GGC GCT TGC AGC ACC GAC AAG

            ↓                       140                                 ↓
pro cys gly lys asp ala gly gly arg val cys thr asn asn tyr cys cys ser lys trp gly ser cys gly ile gly

CCG TGT GGC AAG GAC GCC GGC GGC AGG GTT TGC ACT AAC AAC TAC TGC TGT AGC AAG TGG GGA TCC TGT GGC ATC GGT

          160                                           Ala
pro gly tyr cys gly ala gly cys gln ser gly gly cys asp gly val phe ala glu ala ile ala thr asn ser thr

CCC GGC TAC TGC GGT GCA GGC TGC CAG AGC GGC GGC TGC GAT GGT GTC TTC GCC GAG GCC ATC GCC ACC AAC TCC ACT

leu leu ala glu end

CTT CTC GCA GAA TGA TGA TCTTGCTAATGGTAGTATTGCAACGACGAATAATCCGTGGCAGTTCCATTGCCACGTACGGTTTTCCCTTCACTTAC

TTTTAGTACTAGTACTTAATAATTCTCTAGCTTGCAATATGACATGCAGGTTACTGCAGCAGAAACAAAATATTGCTGTAGTACATGCATGGAAATATTACCG

AGAGAAGTGTTGTGTGGCAATATAGAGTGTACTATAGTTGCCACAAATTAGTGTTCTTGTTATGACCTGCTGTCAGGATGCATGCATGCATCGCTGTTGTAAT

GTTGGAGTACTTCGTGATATCGTTGCAATATATTACCATGAGTCTCACATCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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FIG. 2. Nucleotide sequence of WGA cDNA clone pNVR1. The deduced amino acid residues are shown above the nucleotide triplets. The differences between the deduced amino acid sequence and the published amino acid sequence of isolectin 2 are designated by the amino acid codes above the deduced sequence. The additional differences with isolectin 1 are designated by asterisks. Proline at position 56 is substituted with threonine, and the histidines at positions 59 and 66 are substituted with glutamine and tyrosine, respectively. Previously described differences at positions 150 (25) and 134 (arrows) have been resolved (C. Wright, personal communication). Four termination codons (single underline) and a putative polyadenylylation signal (double underline) are indicated. Fifteen amino acids that extend beyond the carboxyl terminus of the published sequence for WGA are designated by squares. A potential glycosylation site is indicated by dots above the squares.

deduced amino acid sequence with the sequence determined by direct amino acid sequencing of the purified protein (7, 8). The length of the polypeptide derived from the deduced amino acid sequence is 186 amino acids, and the calculated M_r is 18,754. Nevertheless, pNVR1 does not represent the complete coding sequence for WGA. First, the initiating methionine codon is absent from the cDNA. Second, because WGA is synthesized on and translocated across the rough endoplasmic membrane (27), an amino-terminal signal peptide would be expected (28). Third, there may be one or more amino acids at the amino terminus that have not been detected by peptide sequencing because of blockage of the amino terminus (7, 8). The size of the mRNA recognized by clone pNVR1 predicts a full-length cDNA of 1.1 kb.

The DNA sequence of pNVR1 encodes a protein that extends 15 amino acids beyond the carboxyl terminus of the published amino acid sequence and includes a potential site for N-linked glycosylation. Mature WGA is not a glycoprotein, but its precursor form is glycosylated (27). The site of glycosylation probably lies in the 15 amino acid carboxyl-terminal sequence because the only possible site for glycosylation resides in this region. The glycosylated precursor is known to be processed (27) and to accumulate in protein bodies or vacuoles (10, 29). The WGA precursor in the endoplasmic reticulum-associated fraction is 5 kDa larger than the mature WGA (27). The difference in molecular mass between the precursor and mature WGA may be a consequence of the extra 15 amino acids and glycosylation of the

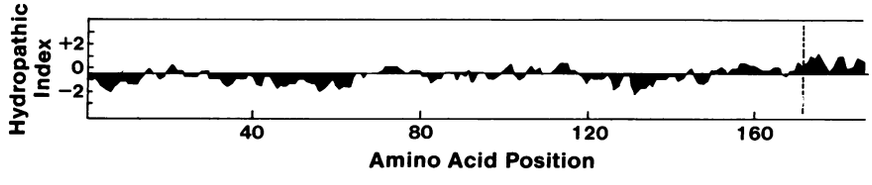

FIG. 3. Hydropathy plot of the protein encoded by cDNA pNVR1. Ordinate, hydropathic index (24); abscissa, amino acid position. The additional 15 amino acids at the carboxyl terminus are right of the broken line.

Table 1. Amino acids at positions in which there are differences between the residues of isolectins 1 and 2 and the protein encoded by pNVR1

Amino acid	Isolectin 1	Isolectin 2	pNVR1
56	Thr	Pro	Pro
59	Gln	His	His
66	Tyr	His	His
93	Ala	Ser	Ala
9	Asn	Asn	Gly
37	Asp	Asp	Asn
53	Ala	Ala	Lys
109	Ser	Ser	Tyr
119	Gly	Gly	Glu
123	Ser	Ser	Asn
171	Ala	Ala	Gly

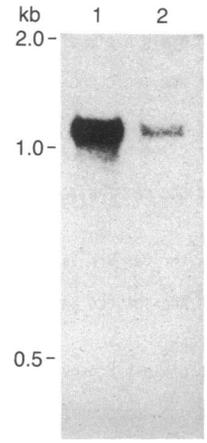

FIG. 4. RNA blot analysis of WGA mRNA levels. Poly(A)$^+$ RNA (1 μg), isolated from embryos excised at 20-day post-anthesis and cultured in the presence (lane 1) and absence (lane 2) of abscisic acid, was separated on a 2% agarose, 6% formaldehyde gel. After transferring the RNA to nitrocellulose, the filter was hybridized to a ^{32}P-labeled DNA insert from pNVR1 under stringent conditions. Positions of DNA M_r markers were obtained from the ethidium bromide-stained portion of the gel.

carboxyl terminus. The hydropathy plot of the amino acid sequence derived from pNVR1 clearly indicates that the carboxyl terminus of the cloned WGA sequence consists of hydrophobic amino acids, which is consistent with the possibility that it is removed post-translationally. Removal of carboxyl-terminal residues was seen during maturation of napin, a rapeseed storage protein (30). It was recently shown that the lectin concanavalin A (Con A), which is not a glycoprotein, is synthesized as a glycosylated precursor (31). Normal transport of this protein is dependent on the presence of the glycan (32). It is interesting that WGA precursor is a biologically active lectin (27), whereas precursor for Con A does not have lectin activity (31). In other words, the loss of the pro-WGA carboxyl-terminal domain does not relate to its ability to bind *N*-acetylglucosamine. Alternatively, cleavage of the carboxyl terminus may occur during the purification of WGA such that the mature protein actually contains 186 amino acids *in vivo*.

Clone pNVR1 mRNA contains four termination codons and a 3′-untranslated region. A potential polyadenylylation signal (AAUAAU) is found in the noncoding region followed by a poly(A) tail. Whereas the consensus sequence for the polyadenylylation signal is very highly conserved in animal systems (AAUAAA), plant mRNAs frequently deviate from this theme (33). The deduced amino acid sequence confirms extensive interdomain homology. The 7-amino acid sequence Gly-Cys-Gln-Asn-Gly-Ala-Cys is found at residues 34–40 and again at residues 120–126. Short repeated stretches of Tyr-Cys-Gly, Ala-Gly-Gly, Gly-Cys-Gln, Cys-Cys-Ser, or Cys-Gly-Gly are found throughout the polypeptide.

Amino acid sequence studies on wheat isolectins 1 (A genome) and 2 (D genome) (5–8) indicate that they differ distinctly in their histidine content: two histidines in isolectin 2 and no histidine in isolectin 1 (8). Because clone pNVR1 was isolated from a cDNA library derived from the tetraploid wheat *T. durum* (AABB), the cDNA clone cannot encode isolectin 2 derived from the D genome. Furthermore, pNVR1 probably does not encode isolectin 1 from the A genome. Isolectin 1 does not contain any histidine, whereas pNVR1 encodes a protein containing two histidine residues. Thus, pNVR1 probably represents isolectin 3 derived from the B genome. Although the amino acid compositions of isolectins 2 and 3 are very similar, eight discrete differences were identified between them. At least four of these differences (residues 9, 53, 93, and 119) are authentic. The x-ray crystallographic data for these four positions in isolectin 2 are definitive, and there is no evidence for heterogeneity in peptide preparations (7). The discrepancies at the remaining

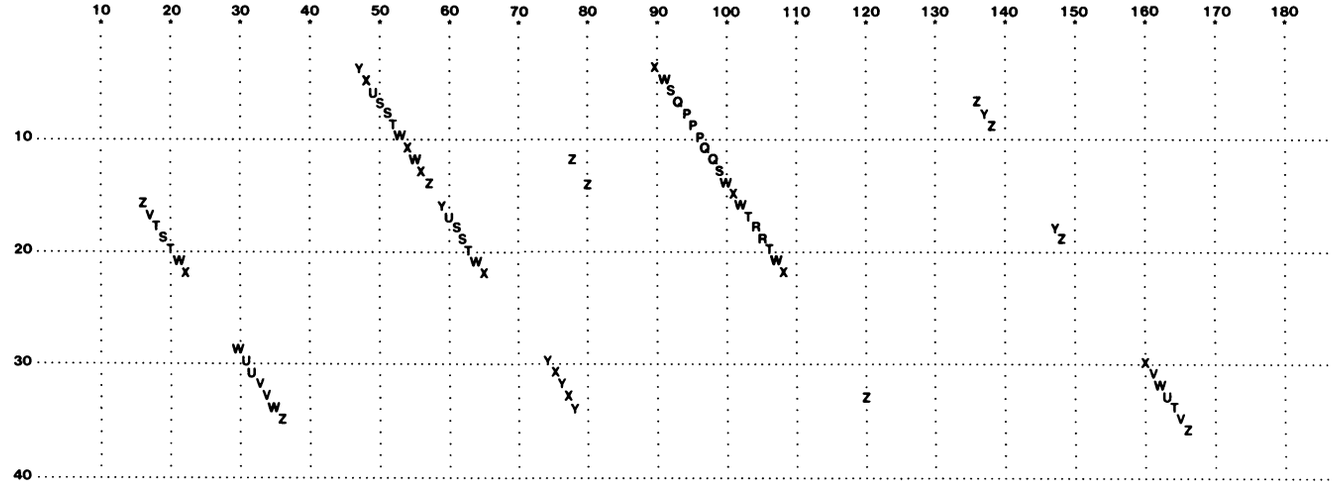

FIG. 5. Amino-acid homology matrix of WGA (*x* axis) and chitinase from *P. vulgaris* (*y* axis). Homology matrices were plotted with the Pustell and Kafatos sequence analysis program (26) using the following parameters: range = 10, scale factor = 0.75, minimum value = 50, compression = 1. Each letter represents homology at that point in the matrix where A = 100%, B = 98%, . . ., Z = 50%. Only homologies in the first 40 amino acids of chitinase are plotted; the remainder of the protein shows no homology with WGA.

four positions (37, 109, 123, and 171) between the deduced amino acids and isolectin 2 could be because of inaccuracies resulting from cross-contamination of the isolectins during fractionation.

Abscisic acid treatment of developing wheat embryos has been shown to affect temporal expression of WGA (12, 15). Using clone pNVR1 as a hybridization probe, we found that abscisic acid treatment of excised wheat embryos modulates mRNA levels for WGA, which is consistent with known effects of abscisic acid on lectin levels (15). Similar results were reported by Williamson *et al.* (21) for the abundant embryo storage protein. It is possible that abscisic acid regulation is based upon changes in the rates of mRNA transcription, turnover, or processing. It also needs to be mentioned that clone pNVR1 may be hybridizing to the mRNAs for isolectins 1 and 2, as well as to the mRNA for isolectin 3 on the RNA blot. Given the similarity of the isolectin sequences, the high-stringency conditions used for hybridization may not have prevented cross-hybridization with mRNAs from related isolectins.

WGA and chitinase are two chitin-binding proteins that are thought to have antimicrobial activity (34). Recently, however, evidence was presented to show that antifungal activity of WGA can result from contamination by chitinase (35). Comparison of amino acid sequences demonstrated a striking homology between the amino terminus of chitinase (16) and four regions of the WGA molecule. The amino acid residues of WGA directly involved in primary sugar-binding sites are tyrosine-73, serine-62, and glutamic acid-115 (36). These three residues are found in the regions of homology between chitinase and WGA. One may speculate that these regions of homology account for the similarity in chitin-binding activity of these proteins and, subsequently, in copurification. Additionally, the sequence homology between WGA and chitinase may be of functional significance.

We thank Dr. Ralph Quatrano, Dr. Rich Meagher, and Dr. W. Michael Ainley for their help in starting this project. We thank Dr. Andrew Hanson and Dr. Michael Mansfield for helpful discussions and for the critical reading of this manuscript. This work was supported by Grant DMB 87-03513 from the U.S. National Science Foundation and by the U.S. Department of Energy Grant under Contract DOE-AC02-76ERO-1338.

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