

# A lectin gene encodes the $\alpha$ -amylase inhibitor of the common bean

(defense proteins/lectin-like protein/plant enzyme inhibitors/seed lectins)

JOAQUIN MORENO\* AND MAARTEN J. CHRISPEELS†

Department of Biology and Center for Molecular Genetics, University of California at San Diego, La Jolla, CA 92093

Communicated by Clarence A. Ryan, July 14, 1989 (received for review April 28, 1989)

**ABSTRACT** An  $\alpha$ -amylase inhibitor that inhibits insect and mammalian  $\alpha$ -amylases, but not plant  $\alpha$ -amylases, is present in seeds of the common bean (*Phaseolus vulgaris*). We have purified the  $\alpha$ -amylase inhibitor by using a selective heat treatment in acidic medium and affinity chromatography with porcine pancreas  $\alpha$ -amylase coupled to agarose. Under sodium dodecyl sulfate gel electrophoresis, the purified inhibitor gave rise to five bands with mobilities corresponding to molecular masses ranging from 14 to 19 kDa. N-terminal sequencing (up to 15 amino acids) of the polypeptides obtained from these bands resulted in only two different sequences matching two stretches of the amino acid sequence deduced from an already described lectin gene [Hoffman, L. M. (1984) *J. Mol. Appl. Gen. 2*, 447–453]. This gene is different from but closely related to the genes that code for phytohemagglutinin, the major lectin of bean. Further evidence based on amino acid composition, identification of a precursor, and recognition of the product of the gene (expressed in *Escherichia coli*) by an anti- $\alpha$ -amylase inhibitor serum confirms that the inhibitor is encoded by this or a closely related lectin gene. This finding assigns a biological function, which has been described at the molecular level, to a plant lectin gene product and supports the defense role postulated for seed lectins. The lack of homology with other families of enzyme inhibitors suggests that this may be the first member of a new family of plant enzyme inhibitors.

Embryo development in plants is accompanied by the accumulation of a number of abundant proteins. Some of these, the storage proteins, play an important role in the nutrition of the seedling, whereas others, such as the lectins and the inhibitors of digestive enzymes of mammals and insects, are considered to be plant defense proteins. Inhibitors of  $\alpha$ -amylases and/or proteases have been classified in nine families according to their sequence identity (1). At least two of these families, the Kunitz trypsin inhibitor and the Bowman-Birk trypsin/chymotrypsin inhibitor, occur in legume seeds. Legume seeds also contain proteins belonging to the family of lectins (i.e., carbohydrate-binding proteins) (2). Although their physiological function remains still unclear (2, 3), plant lectins are widely used as research tools (4) due to their specific carbohydrate-binding properties. Seed lectins are also thought to play a defensive role since some of them are known to be toxic to animal predators (5–7). However, the molecular mechanism of this toxic activity has not been elucidated yet.

An  $\alpha$ -amylase inhibitor ( $\alpha$ AI) has been described in the seeds of several varieties of the common bean, *Phaseolus vulgaris* (8–11). The inhibitor is a thermostable glycoprotein with an apparent molecular mass of 45 kDa, when determined by gel filtration, and it is composed of subunits of about 15 kDa (9, 10). The  $\alpha$ AI complexes at a 1:1 molar ratio with insect and mammalian  $\alpha$ -amylases and some fungal amylolysosidases, but it is inactive against bacterial and higher

plant enzymes (8–11). Comparison of the amino acid composition of the  $\alpha$ AI with those of other inhibitors indicates that the bean inhibitor is unrelated to any of the known families of enzyme inhibitors (1). We have undertaken the characterization of the bean  $\alpha$ AI and report here that this inhibitor is encoded by an already described gene (12, 13) that shows considerable sequence identity with phytohemagglutinin (PHA), the major lectin of bean. We call this gene a lectin gene on the basis of its homology to PHA, although we do not know if the product of this gene is a carbohydrate-binding protein. We suggest that lectins are encoded by families of genes but that all of the products of these genes may not necessarily be carbohydrate-binding proteins.

## MATERIALS AND METHODS

**$\alpha$ AI Extraction.** Mid-mature (18–22 days after pollination) cotyledons of seeds of the common bean (*P. vulgaris* cv. Greensleeves) were homogenized in an ice-cold mortar with 3 ml of 10 mM 2-mercaptoethanol per gram of fresh weight. The homogenate was centrifuged at  $12,000 \times g$  for 10 min and the supernatant fluid was buffered by addition of 0.2 M succinate/0.1 M  $\text{CaCl}_2$ , pH 3.8 (110  $\mu\text{l}/\text{ml}$ ), and heated in a water bath to 70°C for 10 min. The heavy protein precipitate was removed by centrifugation ( $12,000 \times g$  for 10 min) and the clear supernatant was brought to pH 5.6 with NaOH.

**$\alpha$ AI Purification by Affinity Batch Adsorption.** Porcine pancreas  $\alpha$ -amylase (Boehringer Mannheim) was coupled to CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's instructions. The beads with immobilized enzyme were equilibrated with 15 mM succinate/20 mM  $\text{CaCl}_2$ /0.5 M NaCl, pH 5.6 (SS buffer), and the inhibitor extract was added (0.2 ml of extract per ml of beads). The mixture was tumbled at 37°C for 1 hr and then the beads were washed six times with SS buffer (5 ml/ml of beads). The inhibitor was released by tumbling 20 min with 0.12 M sodium citrate, pH 3.0 (1 ml per ml of beads). Then the suspension was centrifuged ( $1500 \times g$  for 5 min) and the supernatant was saved. The citrate washing was repeated and both supernatants were pooled and filtered through a sintered glass funnel.  $\alpha$ AI was concentrated by precipitation with trichloroacetic acid (25% final concentration).

**Purification of the Microsomal Fraction.** Bean cotyledons were homogenized in 0.1 M Tris·HCl/1 mM EDTA with 12% (wt/wt) sucrose, pH 7.8 (1:10, fresh weight to volume ratio). The microsomal fraction was obtained by chromatography on Sepharose 4B as described (14).

**Electrophoresis and Immunoblotting.** NaDodSO<sub>4</sub>/PAGE (15% acrylamide) was performed according to Laemmli (15). Transfer to a nitrocellulose membrane and immunoblot was carried out as detailed in the Bio-Rad technical bulletin. Anti- $\alpha$ AI serum was obtained by injecting a rabbit with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations:  $\alpha$ AI,  $\alpha$ -amylase inhibitor; LLP, lectin-like protein; PHA, phytohemagglutinin.

\*On leave from: Departament Bioquímica i Biologia Molecular, Universitat de València, Burjassot 46100, Spain.

†To whom reprint requests should be addressed.

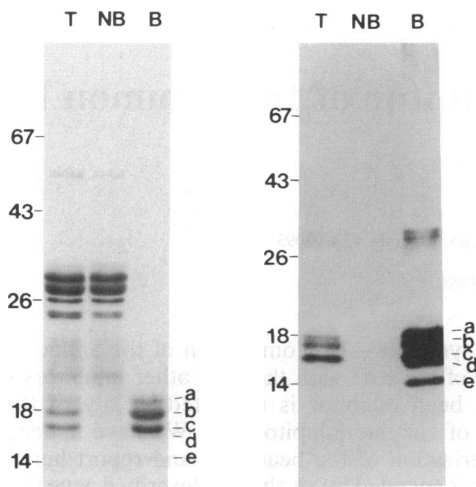


FIG. 1. Purification of the  $\alpha$ AI by affinity batch adsorption.  $\alpha$ AI was extracted from bean cotyledons and adsorbed to porcine pancreas  $\alpha$ -amylase coupled to agarose beads. Total  $\alpha$ AI extract (T) and fractions of material not bound (NB) and bound (B) to  $\alpha$ -amylase-agarose were analyzed by NaDodSO<sub>4</sub>/PAGE followed by Coomassie protein stain (Left) or immunoblotting (Right). Numbers indicate approximate molecular mass in kDa.

purified and chemically deglycosylated  $\alpha$ AI from red kidney beans. Chemical deglycosylation of  $\alpha$ AI was carried out by the method of Edge *et al.* (16). Goat anti-rabbit immunoglobulins coupled to horseradish peroxidase (Bio-Rad) were used as a secondary antibody. Removal of high-mannose glycans was done with endoglycosidase H as described by Trimble and Maley (17).

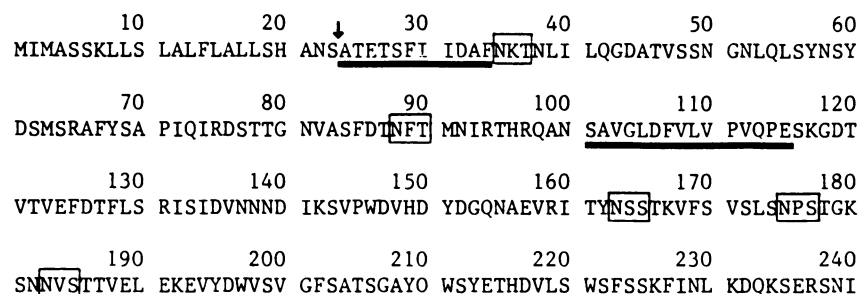
**RNA Extraction and *in Vitro* Translation.** RNA was extracted from bean cotyledons according to Prescott and Martin (18). Poly(A)-RNA was purified by using an affinity paper (Hybond-mAP; Amersham) according to the manufacturer's instructions and translated using the rabbit reticulocyte lysate system (19) in the presence of [<sup>35</sup>S]methionine. The translation product was immunoprecipitated with the anti- $\alpha$ AI serum followed by the addition of immobilized protein A (protein A-Sepharose CL-4; Pharmacia) as de-

scribed (20). The total and immunoprecipitated products were analyzed by NaDodSO<sub>4</sub>/PAGE and fluorography (21).

**Expression of the Lectin-Like Protein (LLP) Gene in *Escherichia coli*.** The LLP coding region [*Bsm* I to *Xba* I fragment of plasmid pPVL134 (12)] was inserted into the multiple cloning site (between *Hind*III and *Xba* I sites) of the pBS(-) vector (Stratagene) in frame with the  $\beta$ -galactosidase sequence. The resulting plasmid was used for *E. coli* (strain DH5 $\alpha$ ) transformation. Parallel controls consisted of cell transformation either with the pBS(-) vector alone (without insert) or with the LLP coding sequence inserted in the reverse orientation (between the *Xba* I and *Sst* I sites). Cloning and transformation were performed by using published procedures (22). Transformants obtained with the three plasmids were grown in liquid medium and the synthesis of the  $\alpha$ -peptide of  $\beta$ -galactosidase or the fusion proteins was induced by adding isopropyl  $\beta$ -D-thiogalactosidase (50  $\mu$ g/ml final concentration). After 16 hr of induction, the cells were harvested, pelleted, and resuspended in 1% NaDodSO<sub>4</sub>/1% 2-mercaptoethanol/6 M urea/10 mM phosphate, pH 7.2 buffer (0.05 ml/ml of original culture). The cell suspensions were incubated at 37°C for 3 hr. The resulting viscous solution was used directly as protein extract. Protein content was determined by the Lowry method (23) after trichloroacetic acid precipitation of a 5- $\mu$ l sample. The extracts were subjected to NaDodSO<sub>4</sub>/PAGE after mixing an aliquot containing 35  $\mu$ g of protein with an equal volume of 30% glycerol/8% NaDodSO<sub>4</sub>/8% 2-mercaptoethanol/40 mM Tris-HCl, pH 6.8 buffer, and boiling for 3 min.

## RESULTS

The  $\alpha$ AI was partially purified from cotyledons of the Green-sleeves cultivar of the common bean by using published procedures involving heat treatment in an acidic medium (9, 10). Further purification was achieved by batch adsorption to porcine pancreas  $\alpha$ -amylase coupled to agarose beads (8). Analysis of the material bound to the amylase beads by NaDodSO<sub>4</sub>/PAGE and Coomassie brilliant blue stain showed five different polypeptides (labeled a-e in Fig. 1) ranging from 14 to 19 kDa [the faint bands seen around 30 kDa are aggregates of these polypeptides (data not shown)]. The



VLNKIL\*

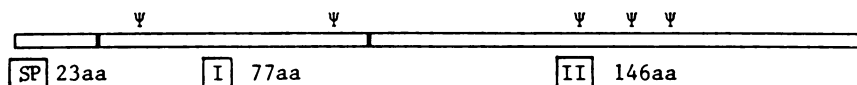


FIG. 2. Comparison of the amino acid sequence of LLP deduced from the nucleotide sequence of the cloned gene (13) with the partial sequence of  $\alpha$ AI. Bars represent the position of the 11 and 15 N-terminal amino acid sequences obtained for the  $\alpha$ AI subunits. The vertical arrow indicates the signal peptide cleavage site deduced by homology with PHA (12) and other lectins (2). N-glycosylation consensus sequences are boxed. The diagram below schematizes the proposed processing of the LLP involving signal peptide removal, glycosylation, and cleavage of the mature polypeptide in two fragments.

immunoblot (developed with a serum obtained from rabbits injected with purified and deglycosylated red kidney bean  $\alpha$ AI) stained only those five bands (Fig. 1), thereby demonstrating the identity between functional and immunological recognition.

To obtain amino acid sequence information, the purified  $\alpha$ AI was subjected to NaDodSO<sub>4</sub>/PAGE and subsequent electrotransfer to a poly(vinylidene difluoride) membrane (24). The bands corresponding to the peptides were cut out and the amino acid sequences were determined. Only two different N-terminal sequences were obtained in agreement with other evidence that some of the  $\alpha$ AI polypeptides are glycoforms (unpublished data). A data bank search of the partial sequences obtained (11 and 15 amino acids long) indicated a 100% match with two stretches of the derived amino acid sequence encoded by a lectin gene from bean (12, 13) (Fig. 2). At the level of the nucleotide sequence, this lectin gene is 65% homologous to PHA, the major seed lectin of bean. At the amino acid level, there is 45% homology between PHA and the encoded protein (LLP); this low value may explain the lack of cross-reactivity of the anti- $\alpha$ AI serum with PHA (the two most abundant polypeptides around 32 kDa in lanes T and NB in Fig. 1). The derived protein sequence possesses five potential N-glycosylation sites (Fig. 1) that could account for the different  $\alpha$ AI glycoforms seen after NaDodSO<sub>4</sub>/PAGE. The N-terminal sequences of the  $\alpha$ AI polypeptides coincide with the N-terminal stretch of LLP (after signal peptide removal at the presumed cleavage site) (12) and with an internal sequence that follows an asparagine residue (Fig. 2), which is a typical processing site for plant seed proteins (25–28). It seems likely, therefore, that  $\alpha$ AI is actually a glycosylated and proteolytically processed form of LLP. Further evidence of the identity between  $\alpha$ AI and LLP can be summarized as follows:

(i) A comparison of the published amino acid composition of the black bean (11) and red kidney bean (9)  $\alpha$ AI with the one of LLP (deduced from the nucleotide sequence) gave values for the Marchalonis–Weltman ( $\Delta$ Q) and difference (DI) indexes that fulfill, respectively, the strong and weak

Table 1. Comparison of the amino acid composition from the black bean and red kidney bean  $\alpha$ AI with the one deduced from the sequence of the LLP gene

Amino acid	% mass		
	AI* black bean	AI† red bean	LLP‡
Asx	19.9	15.6	16.7
Thr	7.3	7.8	7.8
Ser	10.2	13.7	12.0
Glx	10.4	9.2	8.8
Pro	2.8	0.0	2.0
Gly	2.6	2.2	2.1
Ala	3.7	3.1	3.2
Cys	0.2	0.8	0.0
Val	8.0	7.7	8.4
Met	0.9	0.6	1.0
Ile	4.6	4.2	5.9
Leu	5.1	5.0	5.9
Tyr	5.9	8.1	5.3
Phe	7.6	6.9	6.5
His	4.6	1.5	1.7
Lys	0.6	4.6	5.2
Arg	4.6	3.6	4.4
Trp	0.8	5.1	3.0

Difference and Marchalonis–Weltman indices are given in Table 2.

\*From Lajolo and Finardi-Filho (11).

†From Powers and Whitaker (9).

‡Derived from the nucleotide sequence (12).

Table 2. Difference (DI) and Marchalonis–Weltman ( $\Delta$ Q) indexes derived from the data of Table 1

	$\alpha$ AI red bean		LLP	
	DI	$\Delta$ Q	DI	$\Delta$ Q
$\alpha$ AI black bean	13.72*	79.2	10.97*	45.9*
$\alpha$ AI red bean	—	—	7.76†	22.8†

\*Fulfillment of the weak test of relatedness (29).

†Fulfillment of the strong test of relatedness (29).

tests of protein relatedness of Cornish-Bowden (29) (Tables 1 and 2). In fact, these values are more fitting than those obtained comparing directly the composition of black and red kidney beans. This result indicates not only a close relationship between the proteins but also very few amino acids, if any, are lost during the putative processing of the LLP to give the  $\alpha$ AI.

(ii) We isolated poly(A)-RNA from mid-mature beans (about 20 days after pollination, a time when  $\alpha$ AI accumulates rapidly in the seed), and *in vitro* translation of the RNA with [<sup>35</sup>S]methionine and immunoprecipitation of the translation product with anti- $\alpha$ AI serum, followed by NaDodSO<sub>4</sub>/PAGE and fluorography, gave a single polypeptide of 28 kDa (Fig. 3a). This indicates that  $\alpha$ AI is synthesized as a precursor, the size of which matches the one expected for LLP (including signal peptide).

(iii) An immunoblot of an endoplasmic reticulum-rich microsomal fraction from bean cotyledons showed the presence of stained polypeptides of 30–35 kDa reacting with the anti- $\alpha$ AI serum (Fig. 3b). Treatment of the dissolved microsomes with endo- $\beta$ -N-acetylglucosaminidase H (a glycosidase that removes high-mannose glycans) reduced the size of the immunoreactive polypeptide to 25 kDa (Fig. 3b), which matches the size expected for deglycosylated LLP (after signal peptide removal). We presume that the polypeptide in the microsomal fraction is a biosynthetic precursor, since all other abundant seed glycoproteins have been shown to be synthesized on the endoplasmic reticulum and are transported to the vacuoles in a Golgi-mediated process. The

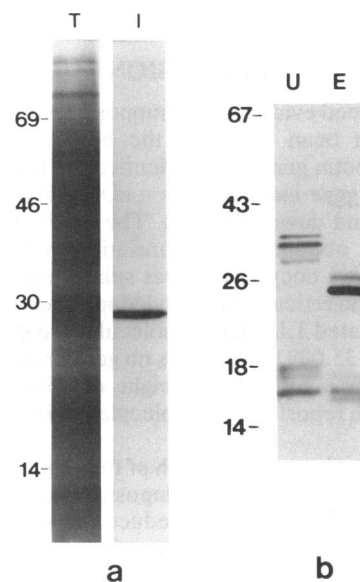


FIG. 3. Evidence for a high molecular weight precursor of  $\alpha$ AI. (a) *In vitro* translation of poly(A)-RNA isolated from mid-mature (20 days after pollination) beans. Lanes show NaDodSO<sub>4</sub>/PAGE fluorographies of total translation product (T) and polypeptides immunoprecipitated with anti- $\alpha$ AI serum (I). (b) Immunoblot of a microsomal fraction from bean cotyledons, untreated (U) or digested with endo- $\beta$ -N-acetylglucosaminidase H (E). Numbers indicate approximate molecular mass in kDa.

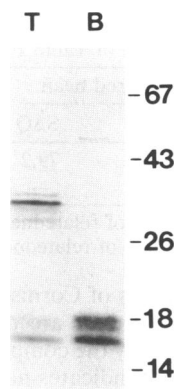


FIG. 4. Adsorption of polypeptides from the microsomal fraction to porcine pancreas  $\alpha$ -amylase coupled to agarose: Immunoblots of total microsomal extract (T) and fraction bound to immobilized  $\alpha$ -amylase (B). Numbers indicate approximate molecular mass in kDa.

putative precursor did not bind to porcine pancreas  $\alpha$ -amylase coupled to agarose (Fig. 4).

(iv) We expressed the LLP gene in *E. coli* by cloning it into a pBS(-) vector plasmid in frame with the  $\beta$ -galactosidase (*lacZ*) gene. Expression of the fusion protein (containing 18 amino acids of the  $\alpha$ -peptide of  $\beta$ -galactosidase at the amino end of the complete LLP) was induced with isopropyl  $\beta$ -D-thiogalactoside, and total protein extracts from the transformed *E. coli* cells were analyzed by NaDodSO<sub>4</sub>/PAGE and immunoblotting with anti- $\alpha$ AI serum. As shown in Fig. 5, the lane corresponding to the in-frame fusion of LLP with  $\beta$ -galactosidase contains several polypeptides cross-reacting with the anti- $\alpha$ AI antibodies. It is likely that the band of highest molecular mass (near 27 kDa) corresponds to the fusion protein, whereas the others represent endogenous degradation products. No signals appeared in the two controls: *E. coli* transformed with the pBS(-) vector or with a fusion of the  $\beta$ -galactosidase gene with the LLP coding sequence in reverse orientation.

## DISCUSSION

We have obtained evidence that supports the identification of the  $\alpha$ AI from bean seeds as the hitherto undetermined product of a lectin gene that is identical with or very closely related to the gene isolated from a cDNA bean seed library by Hoffman and coworkers (12). The product of this lectin gene was first obtained after translation of bean cotyledon RNA in *Xenopus* oocytes and was subsequently detected in the endoplasmic reticulum of cotyledons of developing beans (30). Glycosylated LLP has a molecular weight on NaDodSO<sub>4</sub>/PAGE of 35,000 but behaves on gel filtration columns as a protein with a molecular weight of 45,000. This is in agreement with reports on the molecular weight of the  $\alpha$ AI (9, 10).

In addition to the perfect match of the sequenced stretches and the global amino acid composition of  $\alpha$ AI with the characteristics of the LLP as deduced from the nucleotide sequence of the gene, there is also evidence of a precursor of the  $\alpha$ AI having the same size of the LLP and of cross-reactivity of the anti- $\alpha$ AI serum with the LLP gene product expressed in *E. coli*. Hence, we believe that the  $\alpha$ AI of bean is the product of the LLP gene. We cannot exclude the possibility that the  $\alpha$ AI is encoded by a closely related lectin gene that has not yet been discovered. We consider this an unlikely possibility since the LLP gene is abundantly expressed at the mRNA level (12, 13) and at the protein level (30), and the  $\alpha$ AI is also an abundant protein, making up

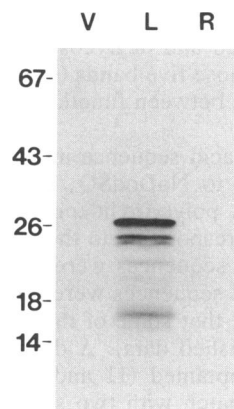


FIG. 5. Expression of the LLP gene in *E. coli*. Lanes show immunoblot of total protein extracts of *E. coli* transformed with the pBS(-) vector alone (V) or including the LLP coding region inserted either in frame with the  $\beta$ -galactosidase sequence of pBS(-) (L) or in the reverse orientation (R). Thirty-five micrograms of total protein was loaded in each lane. Numbers indicate approximate molecular mass in kDa.

several percent of the total protein in mature seeds (9–11). Expression of the LLP gene in *E. coli* did not result in the formation of active protein. Confirmation that the  $\alpha$ AI is encoded by the LLP gene and not by a closely related lectin gene must await the expression of this gene in the seeds of transgenic plants. However, this confirmation is not necessary to reach the conclusion that  $\alpha$ AI is encoded by a lectin gene.

Since their discovery a century ago, scientists have searched for the role of lectins, especially seed lectins. Although many roles have been proposed (2, 3), the idea of lectins as defense proteins of the seed still has the most credibility. This idea recently received further support from the demonstration that certain wild relatives of the common bean contain arcelin, another lectin-like protein that is encoded by a gene that has 78% identity with PHA (identity at the nucleotide level). Arcelin strongly retards the development of seed-eating bruchid beetles (7). Like  $\alpha$ AI, it is also the product of a lectin gene, although it also may not be a true lectin—that is, it may not be a carbohydrate-binding protein. We therefore propose that seed lectins are encoded by gene families, some members of which encode the known seed lectins (e.g., PHA), whereas other members encode proteins that may or may not have carbohydrate-binding properties. We consider all of these genes to be lectin genes. With respect to the PHA gene family, all members appear to be involved in plant defense against insects. Our identification of an enzyme inhibitor that discriminates between endogenous (plant) and foreign amylases as the product of a lectin gene also adds support to the hypothesis that lectin gene products are plant defense proteins. There is evidence of a close linkage on the chromosome between the four lectin genes (PHA-E, PHA-L, arcelin, and the LLP gene) described in bean (31, 32). Therefore, it seems likely that they arose from duplication of an ancestral gene and that the gene products may have evolved specific functions within the general context of plant defense. So the present lectins of the bean display toxic (PHA, arcelin) as well as enzyme inhibitory ( $\alpha$ AI) activity in a phenomenon of functional convergence with other families of inhibitors that are also present in the bean (33). Since the 35-kDa precursor of  $\alpha$ AI present in the microsomal fraction does not bind to immobilized porcine pancreas  $\alpha$ -amylase (Fig. 4), it follows that proteolytic processing and/or glycan modification in the Golgi are necessary for the acquisition of the inhibitory activity. It seems likely

that during evolution this activity appeared as a result of a fortunate processing of a mutated lectin gene.

We thank J. R. Whitaker (University of California, Davis) for the gift of a sample of purified red kidney bean  $\alpha$ AI, M. Williamson for the amino acid sequencing, Brian W. Tague for doing the gene bank amino acid sequence search, L. Hoffman (Agrigenetics, Madison, WI) for the gift of the plasmid pPVL134, and A. Vitale for providing unpublished information on LLP. One of the authors (J.M.) was the recipient of a fellowship from the Conselleria de Cultura, Educacio i Ciencia de la Generalitat Valenciana. Research in the laboratory of M.J.C. is supported by grants from the National Science Foundation, the U.S. Department of Energy, and the U.S. Department of Agriculture.

1. Garcia-Olmedo, F., Salcedo, G., Sanchez-Monge, R., Gomez, L., Royo, J. & Carbonero, P. (1987) *Oxford Surv. Plant Mol. Cell Biol.* **4**, 275–334.
2. Liener, I. E., Sharon, N. & Goldstein, I. J. (1986) *The Lectins* (Academic, Orlando, FL).
3. Sharon, N. & Lis, H. (1987) *Trends Biochem. Sci.* **12**, 488–491.
4. Lis, H. & Sharon, N. (1986) *Annu. Rev. Biochem.* **55**, 35–67.
5. Puzstai, A., Clarke, E. M. W. & King, T. P. (1979) *Proc. Nutr. Soc.* **38**, 115–120.
6. Gatehouse, A. M. R., Dewey, F. M., Dove, J., Fenton, K. A. & Pusztai, A. (1984) *J. Sci. Food Agric.* **35**, 373–380.
7. Osborn, T. C., Alexander, D. C., Sun, S. S. M., Cardona, C. & Bliss, F. A. (1988) *Science* **240**, 207–210.
8. Marshall, J. J. & Lauda, C. M. (1975) *J. Biol. Chem.* **250**, 8030–8037.
9. Powers, J. R. & Whitaker, J. R. (1977) *J. Food Biochem.* **1**, 217–238.
10. Pick, K. H. & Wöber, G. (1978) *Hoppe-Seyler's Z. Physiol. Chem.* **359**, 1371–1377.
11. Lajolo, F. M. & Finardi-Filho, F. (1985) *J. Agric. Food Chem.* **33**, 132–138.
12. Hoffman, L. M., Ma, Y. & Barker, R. F. (1982) *Nucleic Acids Res.* **10**, 7819–7828.
13. Hoffman, L. M. (1984) *J. Mol. Appl. Genet.* **2**, 447–453.
14. Sturm, A., Van Kuik, J. A., Vliegenthart, J. F. G. & Chrispeels, M. J. (1987) *J. Biol. Chem.* **262**, 13392–13403.
15. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
16. Edge, A. S. B., Faltynek, C. R., Hof, L., Reichert, L. E., Jr., & Weber, P. (1981) *Anal. Biochem.* **118**, 131–137.
17. Trimble, R. B. & Maley, F. (1984) *Anal. Biochem.* **141**, 515–522.
18. Prescott, A. & Martin, C. (1987) *Plant Mol. Biol. Rep.* **4**, 219–224.
19. Jackson, R. J. & Hunt, T. (1982) *Methods Enzymol.* **96**, 50–74.
20. Faye, L. & Chrispeels, M. J. (1987) *Planta* **170**, 217–224.
21. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
24. Matsudaira, P. (1987) *J. Biol. Chem.* **262**, 10035–10038.
25. Higgins, T. J. V., Chandler, P. M., Zurawski, G., Button, S. C. & Spencer, D. (1983) *J. Biol. Chem.* **258**, 9544–9549.
26. Carrington, D. M., Auffret, A. & Hanke, D. E. (1985) *Nature (London)* **313**, 64–67.
27. Halling, K. C., Halling, A. C., Murray, E. E., Ladin, B. F., Houston, L. L. & Weaver, R. F. (1985) *Nucleic Acids Res.* **13**, 8019–8033.
28. Borroto, K. & Dure, L., III. (1987) *Plant Mol. Biol.* **8**, 113–131.
29. Cornish-Bowden, A. (1980) *Anal. Biochem.* **105**, 233–238.
30. Vitale, A., Zoppe, M. & Bollini, R. (1987) in *Plant Membranes: Structure, Function, Biogenesis*, eds. Leaver, C. & Sze, H. (Liss, New York), pp. 359–368.
31. Osborn, T. C., Blake, T., Gepts, P. & Bliss, F. A. (1986) *Theor. Appl. Genet.* **71**, 847–855.
32. Staswick, P., Chappell, J., Voelker, T., Vitale, A. & Chrispeels, M. J. (1986) in *Molecular Biology of Seed Storage Proteins and Lectins*, eds. Shannon, L.M. & Chrispeels, M. J. (Am. Soc. Plant Physiol., Rockville, MD), pp. 107–115.
33. Wilson, K. A. & Laskowski, M., Sr. (1973) *J. Biol. Chem.* **248**, 756–762.