Isolation and Characterization of Subunits of DB58, a Lectin from the Stems and Leaves of *Dolichos biflorus*†

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ABSTRACT: The DB58 lectin of the stems and leaves of *Dolichos biflorus* is a heterodimer composed of two closely related subunits, α and β. These subunits were dissociated from one another in urea and isolated by high-performance anion-exchange chromatography. Steric exclusion chromatography of the isolated subunits in 6 M guanidine hydrochloride showed molecular weights of 30 900 and 29 800 for the α and β subunits, respectively. The subunits have very similar amino acid compositions and are glycosylated at each of their two N-glycosylation consensus sites. Each of the subunits had weak carbohydrate binding activity. Reverse-phase chromatography of tryptic digests of the subunits showed identical peptide maps with the exception of peaks identified as COOH-terminal peptides. Analyses of these peptides, COOH-terminal amino acid analyses, and the small differences in amino acid composition between the 2 subunits establish that the β subunit differs from the α subunit by the absence of 11 or 12 amino acids from its COOH terminus. This structural difference, combined with information from previous biosynthetic studies, establishes that the β subunit is derived from the α subunit by posttranslational proteolytic modification at the COOH terminus. The heterogeneity in the extent of truncation suggests that this conversion occurs by sequential removal of amino acids rather than by endoproteolytic cleavage. The possible physiological significance of this modification is discussed.

Lectins are carbohydrate binding proteins that are widely distributed throughout the plant kingdom, where they have been found to be particularly abundant in the seeds of leguminous plants [for a review, see Etzler (1985)]. Many of these leguminous seed lectins are oligomers composed of two types of subunits (Goldstein & Poretz, 1986), which can display different functional properties (Etzler, 1985). The subunits can originate from different genes, as in the case of the *Phaseolus vulgaris* lectin (Hoffman & Donaldson, 1985), or by proteolytic splitting of a single gene product as has been found in the biosynthesis of favin (Hemperly et al., 1982) and the pea (Foriers et al., 1981) and lentil (Higgins et al., 1983) lectins.

Recent studies on the biosynthesis of the *Dolichos biflorus* seed lectin have suggested differential posttranslational modification as a third type of origin of subunit heterogeneity (Schnell et al., 1987; Quinn & Etzler, 1989). This lectin is a tetramer composed of apparently equal amounts of two types of similar but distinct subunits (Carter & Etzler, 1975a,b) which differ from one another at their COOH termini (Roberts et al., 1982; Schnell & Etzler, 1987). Despite their similarities, only one of the two subunit types has been implicated in the carbohydrate binding activity of this lectin (Etzler et al., 1981).

The stems and leaves of the *Dolichos biflorus* plant contain a lectin, named DB58 (Etzler et al., 1986), that is encoded by a separate gene and has 87% sequence identity with the seed lectin (Schnell & Etzler, 1988; Harada et al., 1990). This lectin is a heterodimer (Talbot & Etzler, 1978) with several differences in carbohydrate binding properties from the α-*N*-acetylgalactosamine-specific seed lectin (Etzler & Kabat, 1970; Etzler & Borrebaeck, 1980; Etzler, 1994). Biosynthetic studies have demonstrated only a single mRNA and translation product for this lectin (Schnell & Etzler, 1988; Schnell et al., 1994), suggesting that the subunit heterogeneity of DB58, like its seed lectin counterpart, may arise by differential posttranslational modification of a single gene product. The present study was undertaken in an effort to determine the structural relationships between these two subunits of DB58.

MATERIALS AND METHODS

Isolation of DB58. DB58 was extracted from stems and leaves of 3 week old *Dolichos biflorus* plants as previously described (Talbot & Etzler, 1978) and isolated by affinity chromatography on hog blood group A+H–Sepharose (Etzler & Borrebaeck, 1980). The lectin was further purified by chromatography on Cibacron blue–Sepharose and subdivided into three distinct ionic fractions by high-performance chromatography on a Bio-Rad DEAE-5PW column. All three of these fractions showed identical subunit profiles on SDS–urea–polyacrylamide gels, and fraction 2 was used as the source for subunit isolation.

Analytical Methods. Protein concentrations were determined by the absorbance at 280 nm ($E^{0.1\%} = 1.38$). Amino acid analyses were performed at the Protein Structure Center on the Davis campus using a Beckman Model 6300 amino acid analyzer after hydrolysis of the samples *in vacuo* in constant-boiling HCl for 24 h at 110 °C. NH_2-Terminal amino acid sequence analyses were also performed at the Protein Structure Center by automated Edman degradation using an ABI 477A gas-phase sequencer. The phenylthiohydantoin amino acid derivatives were separated by on-line reverse-phase HPLC and detected by monitoring the absorbance at 269 nm.

COOH-terminal amino acids were determined by analyses of the amino acids released after digestion of the samples with carboxypeptidase Y (102 units/mg of protein; Takara Biochemical Inc., Berkeley, CA). Protein samples were denatured by heating for 5 min at 100 °C in 0.05 M phosphate

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buffer, pH 6.0, containing 0.1% SDS. After being cooled to room temperature, L-α-amino-β-guanidinopropionic acid hydrochloride (AGPA)[1] (10 nmol/nmol of protein) was added as an internal standard, and the samples were digested at room temperature with carboxypeptidase Y using 0.5 unit of enzyme/nmol of protein in a final volume of 40 μL/nmol of protein. At various time intervals ranging from 5 to 45 min, 40 μL aliquots were removed and heated at 100 °C to stop the reaction. The protein was precipitated from these aliquots with 1% sulfosalicylic acid, and the supernatants were analyzed on a Beckman 6300 amino acid analyzer.

Peptide Maps. Protein samples (750 μg/mL 0.1 M Tris-HCl, pH 8.0) were denatured by heating in a boiling H_2O bath for 2 min and cooled to room temperature. TPCK-treated trypsin (13 600 units/mg; Sigma, St. Louis, MO) was added to these samples in two separate aliquots over an 8 h time period to give a final trypsin:protein ratio of 1:100. The digests were incubated at 37 °C for 20–24 h and then heated in a boiling H_2O bath for 2 min. The digests were passed through 0.45 μm Millipore filters and chromatographed at 0.7 mL/min on a Brownlee Labs 10 μm RP-300 aquapore C-8 reverse-phase column (Applied Biosystems, Santa Clara, CA). The column was run for 5 min in 0.1% TFA, pH 2.2, followed by a 100 min linear gradient to 0.1% TFA in 50% acetonitrile. The absorbance of the eluates was read simultaneously at 230, 280, and 260 nm, and the data were recorded and analyzed using a Maxima software program (Waters Chromatography, Milford, MA).

Deglycosylation. TFMS cleavage of the carbohydrate units from intact lectin was performed for 1 h on ice as described by Karp et al. (1982), using a protein concentration of 0.4 mg/mL. Previous studies have shown that these conditions are sufficient for complete removal of the carbohydrate units from the lectin (Schnell et al., 1994).

Physicochemical Methods. Discontinuous polyacrylamide slab gel electrophoresis was run in 0.1% SDS and 8 M urea as previously described (Carter & Etzler, 1975a). The gels were stained with Coomassie Brilliant Blue (Weber & Osborn, (1969).

High-performance steric exclusion chromatography was conducted in 6 M guanidine hydrochloride in 0.01 M sodium phosphate buffer, pH 6.5, containing 1 mM EDTA (Ui, 1979) using a Bio-Sil TSK-250 column (300 × 7.5 mm) with a 75 × 7.5 mm guard column (Bio-Rad Laboratories, Hercules, CA). All runs were done at ambient temperature at a flow rate of 1 mL/min. The V_0 and V_T of the column were determined using thyroglobulin (670 kDa) and vitamin B-12 (1.35 kDA), respectively. Molecular mass standards were bovine albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), pepsin (35 kDa), carbonic anhydrase (29 kDa), chymotrypsinogen A (25 kDa), trypsinogen (24 kDa), trypsin inhibitor (20.1 kDa), lactalbumin (14.2 kDa), ribonuclease A (13.7 kDa), and insulin (2.9 kDa). The standards were heated at 65 °C in 1 M Tris-HCl, pH 8.0, containing 6 M guanidine hydrochloride, 0.145 M dithiothreitol, and 1 mM EDTA. Those proteins not containing cysteine were prepared by heating at 65 °C for 20 min in the mobile phase buffer.

Carbohydrate Binding Assay. The isolated subunits and intact DB58 were iodinated by the iodine monochloride procedure and assayed for carbohydrate binding as previously described (Etzler, 1994). The iodinated proteins were combined with various concentrations of a blood group A+H–Sepharose conjugate in 10 mM Tris-HCl, pH 7.3, containing 0.05% Tween-20 to give a final volume of 200 μL. After incubation overnight at room temperature, the tubes were microfuged for 2 min, and aliquots of the supernatants were counted in a γ counter. Controls for nonspecific binding were run with various concentrations of an ethanolamine–Sepharose conjugate.

[1] Abbreviations: AGPA, L-α-amino-β-guanidinopropionic acid hydrochloride; TFMS, trifluoromethanesulfonic acid; BGS–Sepharose, a conjugate of blood group A+H substance covalently coupled to Sepharose 4B.

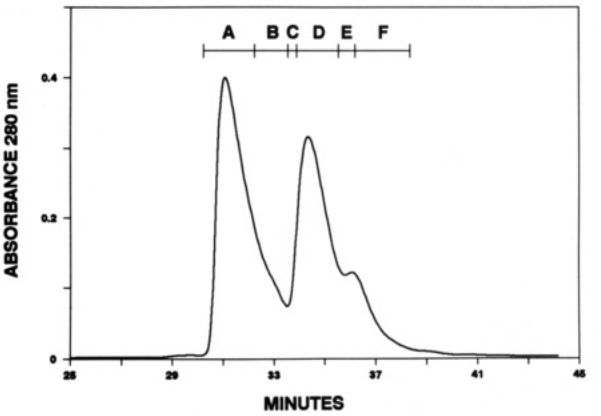

FIGURE 1: Isolation of DB58 subunits by FPLC anion-exchange chromatography. After dissociation in 10 M urea, the subunits were injected onto a DEAE-5PW column equilibrated with buffer A (8 M urea in 0.04 M Tris-HCl, pH 7.3). The column was eluted at 0.8 mL/min with buffer A for 10 min followed by a 50 min linear gradient from buffer A to 0.4 M NaCl in buffer A. Fractions were pooled as indicated by the bars and immediately dialyzed to remove the urea.

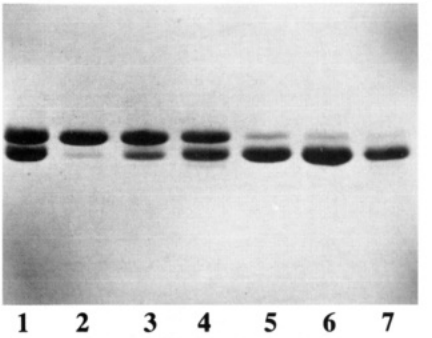

FIGURE 2: SDS–urea–polyacrylamide gel electrophoresis of isolated subunits. Fractions A–F obtained upon ion-exchange chromatography of dissociated DB58 (Figure 1) were analyzed by SDS–urea–PAGE as described in the text. Lane 1, dissociated DB58; lane 2, fraction A; lane 3, fraction B; lane 4, fraction C; lane 5, fraction D; lane 6, fraction E; and lane 7, fraction F.

RESULTS

Isolation of DB58 Subunits. Lyophilized DB58 was added to a freshly made solution of 10 M ultrapure urea in 0.04 M Tris-HCl, pH 7.3, and heated at 85 °C for 2 min. Preliminary experiments showed that this heating step was necessary to dissociate the subunits. The sample was cooled to room temperature, filtered through a 0.45 μm Millipore filter, and immediately chromatographed on an anion-exchange column as shown in Figure 1. Analyses of the pooled fractions by SDS–urea gel electrophoresis (Figure 2) showed that the first peak contains the heavier subunit (designated as α) and the second peak consists primarily of the lighter subunit (designated as β). Fractions from a third peak that was only partially resolved from the trailing edge of the second peak contain a peptide with identical electrophoretic mobility to the β subunit. This fraction was designated as β′ to distinguish it from the

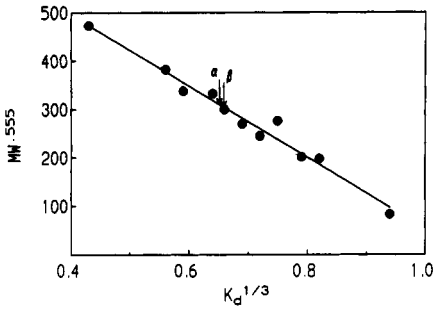

FIGURE 3: Steric exclusion chromatography of isolated α and β subunits. Molecular weight standards (●) were chromatographed on a Bio-Sil TSK-250 column as described in the text, and the distribution coefficients (K_d) were calculated as described by Ui (1979). The molecular weights of the subunits were determined from the positions of their distribution coefficients (arrows) on the linear regression curve which had an r^2 of 0.97.

Table 1: Comparison of Amino Acid Compositions of DB58 Subunits and the Deduced DB58 Sequence

	no. of residues/mol			
amino acid	α subunit	β subunit	β' subunit	deduced[a]
Asx	30.22	28.60	28.60	30
Thr	19.93	20.02	19.83	21
Ser	36.32	35.65	35.75	41
Glx	14.58	14.87	15.25	14
Pro	9.63	9.82	9.53	10
Gly	15.07	15.44	16.20	15
Ala	23.16	22.31	22.49	22
Val	16.97	15.44	16.20	18
Ile	15.25	14.40	14.40	16
Leu	20.02	17.73	17.54	20
Tyr	7.53	6.77	6.67	8
Phe	13.73	13.82	13.92	14
His	2.58	2.58	2.67	3
Lys	8.58	8.40	8.40	9
Arg	7.91	7.05	7.25	8
Trp	nd[b]	nd[b]	nd[b]	4

[a] Number of residues deduced from the genomic DNA sequence (Harada et al., 1990). [b] Not determined.

main β subunit fraction. Subsequent analytic work on the isolated subunits used fraction A for the α subunit, fraction D for the β subunit, and fraction F for the β' subunit.

Characterization of Isolated Subunits. The molecular weights of the isolated subunits were determined by steric exclusion chromatography in the presence of 6 M guanidine hydrochloride. Under these conditions, the α and β subunits were eluted from the column at positions corresponding to M_r's of 30 900 and 29 800, respectively (Figure 3).

The amino acid compositions of the isolated subunits are very similar as shown in Table 1. These values are in close agreement with the number of residues determined from the sequence deduced from the genomic sequence of the coding region of this lectin (Harada et al., 1990). No significant difference was found between the compositions of the β and β' subunits. A comparison of the compositions of the α and β subunits shows that the α subunit contains approximately two more leucine, valine, and asparate/asparagine residues and about one additional serine, alanine, isoleucine, tyrosine, and arginine than the β subunit.

Previous work utilizing the intact lectin and subunits isolated from SDS–urea–PAGE had established that each of the subunits had identical NH_2 termini (Talbot & Etzler, 1978). An attempt was made to determine the COOH termini of these subunits by carboxypeptidase Y digestion. Prior to digestion, the subunits were denatured by heating for 5 min at 100 °C in 0.1% SDS, conditions shown by preliminary

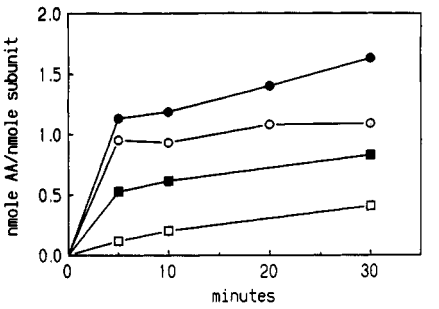

FIGURE 4: COOH-terminal amino acid analyses of isolated α and β subunits. The amino acids released after treatment of the α subunit (circles) and β subunit (squares) with carboxypeptidase Y were measured at various time points. Stoichiometric amounts of leucine (●) and valine (○) were released from the α subunit whereas substoichiometric amounts of leucine (■) and (□) serine were released from the β subunit.

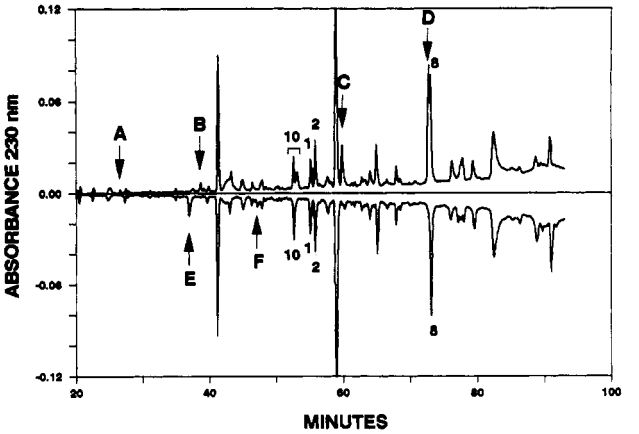

FIGURE 5: Comparison of the tryptic peptide maps of the α and β subunits. After treatment with trypsin, the tryptic peptides from each of the subunits were subjected to reverse-phase chromatography as described in the text. For ease of comparison, different polarities were used for plotting the absorbance of the α subunit peptides (+) and β subunit peptides (–). The letters designate peaks that are unique to either the α or the β subunit. The numbers designate additional peaks that are mentioned in the text.

experiments to improve the accessibility of the COOH termini to the enzyme. As shown in Figure 4, stoichiometric amounts of leucine and valine were rapidly released from the α subunit within the first 5 min of digestion. These residues correspond to the COOH-terminal leucine and penultimate valine previously deduced from the DB58 genomic and cDNA sequences (Schnell & Etzler, 1988; Harada et al., 1990). By 20 min, the digestion had progressed to the release of appreciable quantities of a number of other amino acids, including additional leucine and valine. Digestion of the β subunit under the same conditions progressed at a much slower rate (Figure 4) and yielded only substoichiometric levels of amino acids over the 30 min time period. Leucine was the most prominent amino acid released, followed by serine. Due to insufficient material, carboxypeptidase digests of the β' subunit were analyzed at only two time points. At 10 min, 0.77 nmol of serine and 0.74 nmol of aspartate were released per nanomole of subunit, and these values increased to 0.86 and 0.77 nmol, respectively, by 20 min.

The α and β subunits were heat-denatured and digested for 20–24 h at 37 °C with trypsin at a trypsin:protein ratio of 1:100. Reverse-phase chromatography of these digests yielded very similar peptide maps (Figure 5). Four unique peaks (A–D) were detected in the map of the α subunit, whereas the map of the β subunit showed two unique peaks (E and F). The sequences of these unique peaks were determined by NH_2-

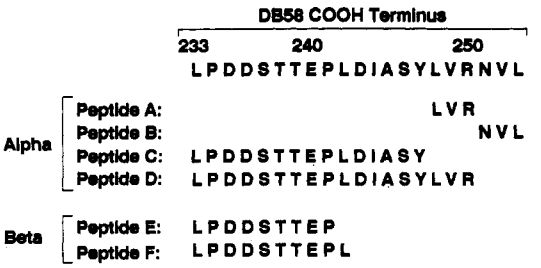

FIGURE 6: Amino acid sequences of tryptic peptides unique to either the α or the β subunit. The amino acid sequences of each of the unique peptides designated by letters in Figure 5 were determined by NH_2-terminal sequencing and/or amino acid analyses. These sequences are aligned with the COOH terminus of DB58 as deduced from the genomic sequence (Harada et al., 1990). Trypsin cleaves the peptide bond between the lysine at residue 232 of that sequence and residue 233 which is the first residue shown.

terminal sequencing and/or amino acid analyses in which the ratios of the amino acids in the isolated peptides were compared to the ratios of amino acids predicted from peptides of the deduced sequence of the entire lectin. These sequences are shown in Figure 6 along with the COOH-terminal sequence of the lectin deduced from its genomic sequence (Harada et al., 1990). All of the unique peptides obtained from the α subunit represent peptides in this COOH-terminal portion of the sequence. Although TPCK-treated trypsin was used in the digests, some cleavage occurred at the tyrosine at position 247. Cleavage at this position also occurred upon trypsin digestion of a synthetic peptide containing this sequence. The sequence of the major unique peptide (E) obtained in the peptide map of the β subunit is equivalent to that of a tryptic peptide that would be obtained from an α subunit truncated by 12 residues, whereas the minor peptide (F) indicates an 11-residue truncation.

The identities of a number of the other peptides in the maps have been established by analyses of tryptic digests of the entire DB58; some of these peptides are designated by small numbers in Figure 5. Both subunits contain peptide 1, ADIQSFSFK, which is the NH_2-terminal tryptic peptide predicted from the lectin sequence. Also of interest is the finding that both subunits contain peptides 2 and 8, which have been identified as the tryptic peptides containing the consensus sites for N-glycosylation. Deglycosylation of intact DB58 prior to tryptic digestion results in a shift in mobility of these two peaks, thus showing that both sites are glycosylated in the lectin. The presence of these two peaks at the retention times of the glycosylated peptides in the maps of both subunits confirms the presence of a carbohydrate unit at each of the two N-glycosylation consensus sites in each of the subunits. The doublet obtained with peak 10 in the α subunit is not always reproducible and has sometimes also appeared in maps of the β subunit. Analyses of both fractions of this peak show a sequence of HIGIDVNSIK which represents positions 138–147 in the lectin sequence.

Carbohydrate Binding Activity. The carbohydrate binding activities of the iodinated α and β subunits and intact DB58 were compared by testing their abilities to bind a conjugate of blood group A+H substance–Sepharose. Very weak binding was detected with the isolated subunits or with a mixture of these subunits. Since the subunits had been iodinated after isolation, it was possible that the iodination had inactivated the subunits. Subunits were therefore isolated directly from active, iodinated DB58 using the procedure established above. Only a small increase in activity was achieved. A comparison of the carbohydrate binding properties of these subunits to

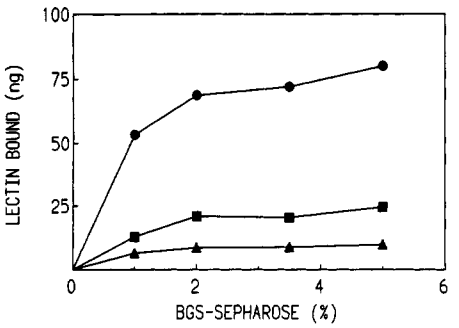

FIGURE 7: Carbohydrate binding activity of isolated subunits and intact DB58. Iodinated DB58 (145.8 ng), α subunit (144 ng), and β subunit (144.9 ng) were added to increasing amounts of BGS–Sepharose in a final volume of 100 μL. After incubation at room temperature overnight, binding was measured as described in the text. (●) DB58; (■) α subunit; (▲) β subunit.

that of intact DB58 is shown in Figure 7. The α subunit had about 30% of the carbohydrate binding activity of the intact lectin, whereas the β subunit showed only about 12% of the activity. Attempts to improve this activity by rapid removal of the urea by spin columns immediately after subunit isolation or by renaturation in the presence of metal ions yielded no significant increase in activity.

DISCUSSION

Subunit heterogeneity is a feature common to a number of legume lectins [for a review, see Goldstein and Poretz, (1986) and Etzler (1992)] and must be considered in studies relating structure to function of these proteins. The isolation of the subunits of DB58 in the present study has enabled the direct characterization of these two very similar polypeptides. COOH-terminal amino acid analyses and analyses of the subunit tryptic peptide maps show that these two subunits differ from one another only at their COOH termini. This finding is supported by a previous study in which only the α subunit reacted in immunoblot analysis with an antiserum made against a synthetic peptide representing the DB58 COOH terminus (Schnell et al., 1994).

The cDNA and genomic DNA for DB58 encode a 275 amino acid sequence, which includes a 22 amino acid NH_2-terminal signal (Schnell & Etzler, 1988; Harada et al., 1990). Previous NH_2-terminal sequence analysis of the intact lectin (Talbot & Etzler, 1978) and the presence of the tryptic peptide ADIQSFSFK in the map of each subunit in the present study verify the removal of the signal sequence from both subunits.

Analyses of the peptides unique to the α subunit and the rapid stoichiometric release of leucine and valine from this subunit upon digestion with carboxypeptidase Y establish that the α subunit represents the entire 253 amino acid sequence of the mature protein which terminates with a leucine and a penultimate valine. This conclusion is also supported by the amino acid composition of this subunit which closely matches the composition predicted from the DNA sequence. The predicted molecular weight of this polypeptide from the DNA sequence was 27 219, a value somewhat lower than the M_r 30 900 value determined in this study. This difference can be attributed to the presence of carbohydrate units at each of the two consensus N-glycosylation sites on this subunit. Glucosamine has been found to be incorporated into each of the two lectin subunits *in vivo* (Schnell et al., 1994), and the mobilities of the tryptic peptides containing the N-glycosylation sites have been found to change upon deglycosylation.

Analyses of the tryptic peptides obtained from the isolated β subunit suggest that it is a mixture of at least 2 polypeptides

differing from the α subunit by the absence of 11 or 12 amino acids from the COOH terminus. The differences in amino acid composition and molecular weight between these two subunits support this conclusion. The predominance of tryptic peptide E over peptide F indicates that a major portion of the β subunit terminates at proline-241 with a minor portion ending at leucine-242. In support of this interpretation is the finding of substoichiometric amounts of leucine released from this subunit upon treatment with carboxypeptidase Y. Although no proline was released, this amino acid is often resistant to cleavage by carboxypeptidase Y due to the type of adjacent amino acids (Hayashi, 1976). Further COOH-terminal heterogeneity is indicated by the partial separation by ion-exchange chromatography of another form of this subunit, designated β'. The release of serine and aspartate upon carboxypeptidase Y digestion of β' suggests that it may terminate at serine-237, which is preceded by an aspartate at positions 235 and 236. The incomplete separation of this β' fraction from the β subunit may account for the low levels of serine released after digestion of the β subunit with carboxypeptidase Y.

Recent *in vivo* pulse–chase labeling studies of DB58 have shown that label is first incorporated into an immunoprecipitable glycosylated polypeptide with an electrophoretic mobility identical to that of the α subunit of the lectin; after a 4 h chase, this label is distributed about equally between immunoprecipitable α and β subunits (Schnell et al., 1994). These results suggested that the β subunit was derived from the α subunit by posttranslational modification. The present data support this interpretation, and the heterogeneity of the COOH terminus of the β subunit suggests that proteolytic conversion occurs by sequential removal of individual amino acids rather than a single endoproteolytic cleavage. This COOH-terminal heterogeneity, coupled with the finding of only one immunoprecipitable *in vitro* translation product and one DB58 mRNA (Schnell & Etzler, 1988; Schnell et al., 1994), helps to rule out the possibility that these different subunits arise by an alternative splicing event.

The derivation of the β subunit of DB58 from its α subunit resembles the origin of subunit II of the *Dolichos biflorus* seed lectin from subunit I (Quinn & Etzler, 1989). COOH-terminal analyses of subunit II, however, have suggested that only 10 amino acids appear to be removed in this conversion (Roberts et al., 1982; Schnell & Etzler, 1987). In the case of both lectins, only about half of the subunits are converted, resulting in the production of heteroligomeric proteins (Carter & Etzler, 1975a; Talbot & Etzler, 1978). Inclusion of protease inhibitors during lectin isolation and variations in time and type of extraction procedures have failed to alter the subunit stoichiometries, leading to the conclusion that the conversion of these subunits occurs *in vivo*. The conversion of only half of these subunits might reflect differences in accessibility to protease after subunit assembly.

In addition to the above *Dolichos biflorus* lectins, several other legume lectins, including concanavalin A and the pea lectin, have been found to undergo the posttranslational removal of several amino acids from their COOH termini during biosynthesis [for a review, see Etzler (1992)]. In concanavalin A, this processing accompanies the posttranslational ligation that results in the formation of the circularly permuted mature form of this lectin (Bowles et al., 1986). It has been suggested that differential cleavage of the COOH terminus of the α chain of the pea lectin may give rise to the two molecular forms of this lectin that are commonly found in peas (Stubbs et al., 1986).

A comparison of the amino acid sequences of a variety of legume lectins [cf. Sharon and Lis (1990)] shows that a number of those legume lectins belonging to the Gal/GalNAc specificity category have extended COOH-terminal sequences. Preliminary computer modeling experiments have shown that although neither the COOH terminus of subunit I of the *Dolichos biflorus* seed lectin nor the COOH terminus of the α subunit of DB58 is capable of extending to the putative carbohydrate binding site of their respective monomers, these COOH termini may be able to interact with the site of the adjacent subunit (Gegg, 1993). Previous studies from our laboratory have shown that the *Dolichos biflorus* seed lectin contains only two carbohydrate binding sites per tetramer and that carbohydrate binding activity is associated only with subunit I (Etzler et al., 1981). A mutant form of this lectin, made by deleting six amino acids from the COOH terminus of subunit I, had no carbohydrate binding activity.[2] It is thus of interest in the present study that both the isolated α and β subunits of DB58 displayed weak carbohydrate binding activity, with the α subunit approximately 2.5 times more active than the β subunit. Although this activity was not increased upon mixing the two subunits, further attempts at subunit recombination under a variety of renaturing conditions should be of interest in defining the individual contributions of these two subunits to the carbohydrate binding activities of this lectin.

In addition to their carbohydrate binding activities, several legume lectins, including the above *Dolichos biflorus* lectins, have been found to have high-affinity hydrophobic sites that can bind adenine and the cytokinin class of plant hormones (Roberts & Goldstein, 1983a,b; Roberts et al., 1986; Gegg et al., 1992). Photoaffinity labeling studies indicate that this binding occurs at a subunit interface (Maliarik & Goldstein, 1988; Gegg & Etzler, 1994). Recent studies on the *Dolichos biflorus* seed lectin implicate the presence of the carboxyl terminus of subunit I in the vicinity of this site and suggest that a heterologous subunit interface may enhance the adenine binding properties of this lectin[2] (Gegg & Etzler, 1994). A heterologous subunit interface has also been implicated in the binding of DB58 to adenine (Gegg & Etzler, 1994). The present characterization of the two subunits of DB58 has provided an insight into the origin and nature of this subunit heterogenity in this lectin and provides a framework for future studies on the relationship of lectin structure to function.

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