NH_2-TERMINAL SEQUENCES OF THE SUBUNITS OF *DOLICHOS BIFLORUS* LECTIN

Marilynn E. ETZLER, Craig F. TALBOT, and Paul R. ZIAYA
Department of Biochemistry and Biophysics, University of California, Davis, California 95616, USA

Received 14 July 1977

1. Introduction

The seeds of the *Dolichos biflorus* plant contain a lectin that has a specificity for terminal nonreducing α-*N*-acetyl-D-galactosamine residues [1,2]. This lectin is a glycoprotein of approx. 110 000 mol. wt and exists in several molecular forms that appear to differ from one another only by slight differences in carbohydrate composition [3].

The predominant form of the lectin is a tetramer composed of two types of subunits, I and II, with mol. wt 27 700 and 27 300, respectively [4]. These subunits have similar amino acid and carbohydrate compositions, show reactions of identity when tested in immunodiffusion against antisera made to either subunit, and have alanine as their NH_2-terminal amino acid [4]. Treatment of the isolated subunits with carboxypeptidase A showed that subunit I has valine or leucine at its COOH-terminal whereas the COOH terminal of subunit II is not cleaved by the enzyme [4]. The above data suggest that subunits I and II may differ from one another only at their COOH-terminal ends and that the NH_2-terminal portions of the subunits may be identical. This hypothesis is supported by the finding that the NH_2-terminal fragments of the subunits, isolated after CNBr treatment of the lectin, have the same electrophoretic mobility whereas the COOH-terminal fragments have different mobilities [5].

In the present paper we report the NH_2-terminal sequences of the first thirty residues of subunits I and II. The identity of these sequences confirms the identity of the NH_2-terminal portions of the subunits. The sequences also show some homologies with NH_2-terminal sequences of other lectins.

2. Materials and methods

The *Dolichos biflorus* lectin was purified by affinity chromatography as previously described [1,2]. Subunits IA and IIA, which are the subunits of the predominant form A of the lectin [3], were isolated by ion-exchange chromatography, after disruption of the lectin in 10 M urea [4]. The isolated subunits were dialyzed against H_2O and lyophilized. Approximately 300 nmol of each subunit were dissolved in 300 μl of 100% formic acid for sequencing.

Automated Edman degradations of each subunit were performed in dimethyallylamine buffer using a Beckman model 890c sequencer with program 102974. The cleaved residues were converted to their phenylthiohydantoin derivatives and identified by gas–liquid chromatography before and/or after trimethylsilylation [6,7], thin-layer chromatography on polyamide sheets [8] and/or by amino acid analysis after back hydrolysis with 6 N HCl containing 0.1% $SnCl_2$ [9]. Amino acid analyses were done on a Durrum analyzer.

3. Results and discussion

Subunits IA and IIa of the *Dolichos biflorus* lectin were each subjected to 30 consecutive cycles of automated Edman degradation, and the cleaved residues were identified as described above. The repetitive yield in each case was approx. 95%. The NH_2-terminal amino acid sequences of the two subunits are identical (fig.1), thus supporting the previous data that suggested that these two similar

	1				5				10				15			
Subunit IA	Ala	Asn	Ile	Gln	Ser	Phe	Ser	Phe	Lys	Asn	Phe	Asn	Ser	Pro	Ser	
Subunit IIA	Ala	Asn	Ile	Gln	Ser	Phe	Ser	Phe	Lys	Asn	Phe	Asn	Ser	Pro	Ser	

	16				20				25							30
Subunit IA	Phe	Ile	Leu	Gln	Gly	Asp	Ala	Thr	Val	—*		—*	Gly	(Lys)	Leu	Gln
Subunit IIA	Phe	Ile	Leu	Gln	Gly	Asp	Ala	(Thr)	Val	(Ser)		(Ser)	Gly	Lys	(Leu)	Glx

Fig.1. NH_2-Terminal amino acid sequences of subunits IA and IIA of the *Dolichos biflorus* lectin. Parentheses indicate residues identified by only one method, *Residues 25 and 26 of subunit IA could not be identified by gas–liquid nor thin-layer chromatography. The aqueous phases of these residues were lost, thus not allowing identification by amino acid analysis after back hydrolysis which was the technique used to identify residues 25 and 26 of subunit IIA.

subunits may differ from one another only at their COOH-terminal ends [4,5]. An identity in NH_2-terminal amino acid sequences has also been reported for the first 10 residues of the two types of subunits of the soybean lectin [10].

Both the soybean and *Dolichos biflorus* lectins are tetramers composed of two types of subunits [3,10]; these two lectins show similar subunit patterns when compared by polyacrylamide gel electrophoresis in a discontinuous anionic buffer system containing SDS and urea [4,10]. The two lectins also have similar carbohydrate binding specificities although they differ in their ranges of specificity [11]. A comparison of the NH_2-terminal sequences of the first 25 amino acids of these lectins (fig.2) shows homologies at positions 1, 5, 6, 7, and 11; in addition to these homologies, the segment of residues 19–24 of the soybean lectin may be homologous to the segment of residues 17–22 of the *Dolichos biflorus* lectin with the exception of a glutamic acid (position 21 in

	1				5				10				15				20				25
D. biflorus	Ala	Asn	Ile	Gln	Ser	Phe	Ser	Phe	Lys	Asn	Phe	Asn	Ser	Pro	Ser	Phe	Ile	Leu	Gln	Gly	Asp	Ala	Thr	Val	Ser
Soybean	—	Glu	Thr	Val	—	—	—	—	Trp	Asn	Lys	—	Val	Pro	Lys	Glu	Pro	Asp	Met	Ile	Leu	Glu	Gly	Asp	Ala	Ile
Kidney bean (R Subunit)	—	Ser	Glu	Thr	—	—	—	—	—	—	Glu	Arg	—	—	—	Glu	Thr	Asn	Leu	—	—	—	Arg	—	—	Ser	—
Kidney bean (L Subunit)	Ser	—	Asp	Ile	Tyr	—	Asn	—	Glu	Arg	—	—	—	Glu	Thr	Asn	Leu	—	—	—	—	—	Arg	—	—	Ser	—
Lentil (β Subunit)	Thr	Glu	Thr	Thr	—	—	—	—	—	—	Ile	Thr	Lys	—	Ser	Pro	Asp	Gln	Gln	Asn	—	Ile	Phe	Gln	Gly	Asp	Gly	Tyr
Pea (β Subunit)	Thr	Glu	Thr	Thr	—	—	—	Leu	Ile	Thr	Lys	—	Ser	Pro	Asp	Gln	Gln	Asn	—	Ile	Phe	Gln	Gly	Asn	Gly	Tyr		
Peanut	—	Glu	Thr	Val	—	—	—	Asn	—	Asn	Ser	—	Ser	Glu	Gly	Asn	Pro	Ala	Ile	Asn	Phe	Gln	Gly	Asp	—	Thr		
Ricin (β Subunit)	—	Asp	Val	Thr	Gln	Asp	Pro	Glu	Pro	Ile	—	Arg	Thr	Val														
Concanavalin A	—	Asp	Thr	Ile	Val	Ala	Val	Glu	Leu	Asp	Thr	Tyr	Pro	Asn	Thr	Asp	—	Gly	Asp	Pro	Ser	Tyr	Pro	His	Ile			

Fig.2. Comparison of NH_2-terminal amino acid sequences of lectins. The sequence of the *Dolichos biflorus* lectin is compared with the sequences of the soy-bean lectin [12], subunits R and L of the kidney bean (PHA) lectin [13], the β subunits of the lentil [12] and pea [14] lectins, the peanut lectin [12], the β subunit of Ricin [15], and concanavalin A [16]. Homologies with the *Dolichos biflorus* lectin are shown by a solid line. Arrows underline additional segments of the *Dolichos biflorus* and soybean lectins that appear to be homologous with each other.

soybean lectin) in place of a glutamine (position 19 in *Dolichos biflorus* lectin).

Of the NH_2-terminal amino acid sequences of other lectins that have been reported [12–16], the sequences of the two subunits, R and L, of the kidney bean lectin (PHA) show the greatest degree of homology with the sequences of the subunits of the *Dolichos biflorus* lectin (fig.2). The R subunit of PHA has homologies with the *Dolichos biflorus* lectin in 13 positions within the first 24 NH_2-terminal residues.

The high degree of homology among the NH_2-terminal sequences of the lectins from *Dolichos biflorus*, soybean, and kidney bean is of particular interest since these plants are closely related, and, although they show some differences in specificity, each of these lectins is inhibited by *N*-acetyl-D galactosamine [1,2,11,17–19].

Among all of the NH_2-terminal sequences of lectins of various specificities shown in fig.2, most lectins show homologies at positions 1, 5, 6, and 11. These homologies support the theory [12] that the genes coding for the various plant lectins may have evolved from a common ancestral gene.

Acknowledgements

We thank Mr A. J. Smith for his valuable technical assistance. This work was supported by US Public Health Service Grant GM 21882 to MEE.

References

[1] Etzler, M. E. and Kabat, E. A. (1970) Biochemistry 9, 869–877.
[2] Etzler, M. E. (1972) Meth. Enzymol. Vol. XXVIII. 340–344.
[3] Carter, W. G. and Etzler, M. E. (1975) J. Biol. Chem. 250, 2756–2762.
[4] Carter, W. G. and Etzler, M. E. (1975) Biochemistry 14, 2685–2689.
[5] Carter, W. G. and Etzler, M. E. (1975) Biochemistry 14, 5118–5122.
[6] Pisano, J. J. and Bronzert, T. J. (1969) J. Biol. Chem. 244, 5597–5607.
[7] Klebe, J. F., Finkbeiner, H. and White, D. M. (1966) J. Am. Chem. Soc. 88, 3390–3395.
[8] Kulbe, K. D. (1974) Anal. Biochem. 59, 564–573.
[9] Mendez, E. and Lai, C. Y. (1975) Anal. Biochem. 68, 47–53.
[10] Lotan, R., Cacan, R., Cacan, M., Debray, H., Carter, W. G. and Sharon, N. (1975) FEBS Lett. 57, 100–103.
[11] Hammarström, S., Murphy, L. A., Goldstein, I. J. and Etzler, M. E. (1977) Biochemistry 16, 2750–2755.
[12] Foriers, A., Wuilmart, C., Sharon, N. and Strosberg, A. D. (1977) Biochem. Biophys. Res. Commun. 75, 980–986.
[13] Miller, J. B., Hsu, R., Heinrikson, R. and Yachnin, S. (1975) Proc. Natl. Acad. Sci. USA 72, 1388–1391.
[14] Van Driessche, Foriers, A., Strosberg, A. D. and Kanarek, L. (1976) FEBS Lett. 71, 220–222.
[15] Li, S. S-l., Wei, C. H., Lin, J-Y. and Tung, T-C. (1975) Biochem. Biophys. Res. Commun. 65, 1191–1195.
[16] Wang, J. L., Cunningham, B. A., Waxdal, M. J. and Edelman, G. M. (1975) J. Biol. Chem. 250, 1490–1502.
[17] Borberg, H., Woodruff, J., Hirschhorn, R., Gesner, G., Miescher, P. and Silber, R. (1966) Science 154, 1019–1020.
[18] Lis, H., Sela, B-A., Sachs, L. and Sharon, N. (1970) Biochim. Biophys. Acta 211, 582–585.
[19] Pereira, M. E., Kabat, E. A. and Sharon, N. (1974) Carbohydr. Res. 37, 89–102.