The amino acid sequence of the α-subunit of a mitogenic lectin from seeds of *Lathyrus odoratus*

Knut Sletten*, Jan Kolberg and Terje E. Michaelsen

**Department of Biochemistry, University of Oslo, P.O. Box 1041, Blindern, Oslo 3* and *Department of Immunology, National Institute of Public Health, Postuttak, Oslo 1, Norway*

Received 29 March 1983; revision received 13 April 1983

We have determined the complete amino acid sequence (54 residues) of the light chain of the mitogenic, glucose- and mannose-specific lectin from the seeds of *Lathyrus odoratus*. The covalent structure was elucidated by Edman degradation on the whole chain as well as on fragments derived from cleaving the chain with BNPS-skatole and of peptides derived after tryptic digestion of the citraconylated chain. The amino acid sequence revealed a high homology to the light chains of lectins from *Pisum sativum* and *Lens culinaris*.

Lathyrus odoratus *lectin* α *Subunit* *Amino acid sequence*

1. INTRODUCTION

Lectins constitute a special group of sugar-binding proteins of non-immune origin that agglutinate cells and precipitate glycoconjugates. They occur in many types of organisms and display a wide variety of unique and interesting chemical and biological effects [1,2]. The lectins are being used as tools in studies of the carbohydrate moieties of cell surfaces, in the characterization of cellular reactions in immunology and in isolation and characterization of glycoproteins. It is therefore of great importance to elucidate molecular properties of the lectins in order to clarify their structures and biological functions. The isolation and characterization of a mitogenic lectin from seeds of sweet pea, *Lathyrus odoratus*, has been reported [3–5]. The glucose and mannose specific *L. odoratus* lectin was found to be composed of 2 light chains (α, M_r ~4400) and 2 heavy chains (β, M_r ~19000). This subunit structure is similar to that of *Lens culinaris, Vicia faba* and *Pisum sativum* lectins [6–8], a similarity which was confirmed by the data obtained from amino-terminal analyses [4]. This relationship is also in accordance with plant taxonomy since the genera *Lathyrus, Vicia* and *Pisum* belong to the Vicieae tribe of the Leguminosae family. In this report, the covalent structure of the α subunit of the *L. odoratus* lectin is being presented, and the homology to α-subunits from other 2-chain lectins is discussed.

2. MATERIALS AND METHODS

The seeds of *Lathyrus odoratus* were purchased from Norwegian Seeds A/S. The lectin was isolated by affinity chromatography on Sephadex G-100 [4]. The subunits were separated by gel filtration on a Sephadex G-100 column equilibrated with 2 M acetic acid.

2.1. *Citraconylation*

The α-subunit was citraconylated in 5 μM borate buffer containing 6 M guanidine–HCl and essentially as in [9].

2.2. *Proteolytic digestion*

The citraconylated α-subunit was digested with TPCK-treated trypsin (Worthington) for 2 h at 37°C in 0.1 M NH_4HCO_3 (pH 8.5). The digestion was stopped by addition of formic acid to pH ~2.

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

Under these conditions the C-terminal peptide (Tc-2) precipitated out, whereas the N-terminal peptide (Tc-1) stayed in solution. Carboxypeptidase A and B digestion of the BNPS-3 fragment was performed as in [10].

2.3. *Cleavage with BNPS-skatole*

α-Subunit (5 mg) was cleaved with BNPS-skatole [3-bromo-2-(2-nitrophenylsulfenyl) skatole], essentially as in [11].

2.4. *Purification of peptides*

Peptides obtained from cleavage with BNPS-skatole were purified by gel filtration on a column (1 × 106 cm) of Sephadex G-50 in 10% acetic acid and by high-performance liquid chromatography (HPLC). A reversed phase column (4.6 × 250 cm) with LiChrosorb RP 18 (10 μm) packing (Altex) was developed with a linear gradient over 45 min from 0.1% orthophosphoric acid and acetonitrile from 0–50%. A constant flow rate of 1 ml/min was maintained as in [12]. The HPLC instrumentation was an Altex Scientific Instrument, model 312 with a Uvikon LCD 725 variable wavelength detector and with a W + W Recorder 600 (Kontron).

2.5. *N-Terminal sequence analysis*

Automated sequence analyses were performed with a JEOL JAS-47K sequence analyser as in [10].

2.6. *Amino acid analysis*

The amino acid composition of the subunit and the peptides were determined as in [10].

3. RESULTS AND DISCUSSION

The purity of the α-subunit was checked by SDS–polyacrylamide gel electrophoresis and by N-terminal analysis [4]. Automatic Edman degradation gave only valine in position 1 in a yield of ~70% of the applied material. However, after step 2, the yield dropped to ~20%. A rather abnormal streaking effect of the PTH-derivative from step 2 was observed on the thin-layer chromatogram. This observation has not earlier been noticed and it thus raises the question if the threonine residue in position 2 could be a modified one. Amino acid composition of the protein, as well as peptides obtained from the N-terminal region (tables 1 and 2) showed, however, no abnormalities when analysed

Table 1

Amino acid composition of the α-subunit as well as of tryptic peptides obtained after citraconylation of the subunit

Amino acid	α-Subunit	Tc-1	Tc-2
Asp	2.42 (2)	2.0 (2)	0.4
Thr	5.14 (5)	1.4 (2)	3.0 (3)
Ser	6.60 (7)	0.5 (1)	6.0 (6)
Glu	5.99 (6)	2.1 (2)	3.7 (4)
Pro	2.0 (2)	1.9 (2)	–
Gly	5.21 (5)	–	5.3 (5)
Ala	4.00 (4)	–	4.0 (4)
Val	6.59 (7)	4.4 (6)	1.3 (1)
Ile	0.98 (1)	–	1.2 (1)
Leu	4.11 (4)	1.9 (2)	2.2 (2)
Tyr	1.00 (1)	0.7 (1)	–
Phe	3.45 (3)	–	3.1 (3)
His	2.03 (2)	–	1.8 (2)
Lys	1.90 (2)	1.0 (1)	1.1 (1)
Arg	1.05 (1)	0.9 (1)	–
Trp	(2)	(1)	(1)
Residues	1–54	1–21	22–54

Residues in brackets are from sequence determinations

Table 2

Amino acid composition of peptides obtained after cleavage of the α-subunit with BNPS-skatole

AA	BNPS-1	BNPS-1 + 2	BNPS-3
Asp	2.0 (2)	1.8 (2)	–
Thr	2.0 (2)	3.2 (4)	1.0 (1)
Ser	1.1 (1)	4.0 (3)	3.6 (4)
Glu	2.1 (2)	3.9 (4)	1.8 (2)
Pro	1.9 (2)	1.2 (2)	–
Gly	0.1	3.1 (2)	3.0 (3)
Ala	0.1	3.6 (4)	–
Val	3.1 (5)	3.3 (7)	–
Ile	–	0.8 (1)	–
Leu	1.9 (2)	2.7 (3)	1.0 (1)
Tyr	0.4 (1)	0.4 (1)	–
Phe	–	1.2 (2)	0.9 (1)
His	–	0.9 (1)	0.9 (1)
Lys	1.0 (1)	1.0 (1)	1.0 (1)
Arg	–	0.6 (1)	
Trp	(1)	(2)	
Residues	1–19	1–40	41–54

Residues in brackets are from sequence determinations

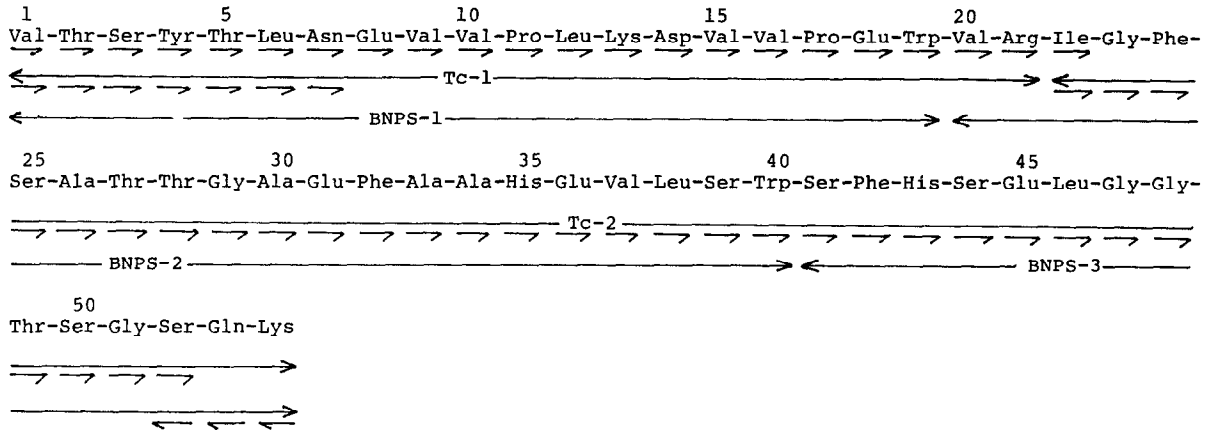

Fig.1. The amino acid sequence of the α-subunit of the lectin from *L. odoratus* seeds. The various peptides are indicated by double-headed arrows. Half-arrows pointing to the right indicate PHT-amino acid derivatives identified by Edman degradation. Half arrows pointing to the left are amino acid residues identified by carboxypeptidase digestion. Tc, peptides obtained after tryptic digestion of citraconylated protein. BNPS, fragments obtained after cleavage at the tryptophan residues with BNPS-skatole.

as standard runs on the amino acid analyser.

The covalent structure of residues 1–50 was established by cleaving the citraconylated protein by trypsin at the only arginine residue, followed by N-terminal analyses of the separated fragments (table 1, fig.1). To confirm the sequence and to determine the amino acid sequence in the C-terminal region, the protein was cleaved by BNPS-skatole. Three fragments were separated by gel filtration and by high performance liquid chromatography. Amino acid analyses of the purified fragments (table 2), and digestion of the BNPS-3 fragment with carboxypeptidase A and B, resulted in the complete structure of the α-subunit. The polypeptide was found to consist of 54 amino acid residues, a number slightly higher than that found in other α-subunits from 2-chain lectins. The M_r calculated from the sequence (5817) deviates, however, from that found by sedimentation equilibrium analysis [3].

When a comparison of the amino acid sequences of different α-subunits from 2-chain lectins is being made, using residues 1 through 51, the subunit from *L. odoratus* is most similar to that from *P. sativum* and *L. culinaris* [13]. Amino acid residue alterations between *L. odoratus* and that from *P. sativum* are found in positions 7, 8, 11, 32, 47 and 51 and between *L. odoratus* and that from *L. culinaris,* in positions 35, 38, 43, 48 and 51. In the first comparison, 3 replacements occur in the N-terminal region and 3 in the C-terminal region, whereas in the second comparison, all 6 replacements occur in the C-terminal region.

ACKNOWLEDGEMENTS

The work was supported by grant from the Norwegian Research Council for Science and the Humanities. We thank Mrs J. Juul for excellent technical assistance.

REFERENCES

[1] Lis, H. and Sharon, N. (1981) in: The Biochemistry of Plants (Marcus, A. ed) vol.6, pp.371–447, Academic Press, New York.
[2] Brown, J.C. and Hunt, R.C. (1978) Int. Rev. Cytol. 52, 277–349.
[3] Kolberg, J. and Michaelsen, T.E. (1979) Acta Pathol. Microbiol. Scand. Sect. C, 87, 275–279.
[4] Kolberg, J., Michaelsen, T.E. and Sletten, K. (1980) FEBS Lett. 117, 281–283.
[5] Kolberg, J. and Sletten, K. (1982) Biochim. Biophys. Acta 704, 26–30.
[6] Foriers, A., De Neve, R., Kanarek, L. and Strosberg, A.D. (1978) Proc. Natl. Acad. Sci. USA 75, 1136–1139.
[7] Hopp, T.P., Hemperly, J.J. and Cunningham, B.A. (1982) J. Biol. Chem. 257, 4473–4483.

[8] Richardson, C., Behnke, W.D., Freisheim, J.H. and Blumenthal, K.M. (1978) Biochim. Biophys. Acta 537, 310–319.
[9] Jaton, J.C. (1974) Biochem. J. 141, 1–13.
[10] Sletten, K., Natvig, J.B., Husby, G. and Juul, J. (1981) Biochem. J. 195, 561–572.
[11] Fontana, A. (1972) Methods Enzymol. 19, 419–423.
[12] Fullmer, C.S. and Wasserman, R.H. (1979) J. Biol. Chem. 254, 7208–7212.
[13] Baumann, C.M., Strosberg, A.D. and Rüdinger, H. (1982) Eur. J. Biochem. 122, 105–110.