SUBUNIT STRUCTURE AND N-TERMINAL SEQUENCES OF THE *LATHYRUS ODORATUS* LECTIN

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1. Introduction

A mitogenic lectin (LATH) from the seeds of sweet pea (*Lathyrus odoratus*) belonging to the *Leguminosae* family, has been isolated by ammonium sulphate precipitation and subsequent ion exchange chromatography [1]. SDS gel electrophoresis of the LATH lectin revealed one major band (M_r 19 000) and a faint band of smaller molecular weight. The M_r of the untreated lectin was estimated to be 37 000 by gel filtration under neutral conditions. It was suggested that the lectin was composed of two subunits of M_r 19 000, presuming that the faint band was a contaminant [1]. Its mitogenic activity was strongly inhibited by α-methyl-D-mannoside [2], indicating sugar specificities similar to that of Con-A, pea and lentil lectins.

Other studies have shown that glucose–mannose-binding leguminous lectins such as pea and lentil are composed to two heavy (β) and two light (α) chains [3], whereas Con-A with similar sugar specificity is composed of monomers [4]. This work was undertaken to study the subunit structure and the N-terminal sequences of the LATH lectin.

2. Materials and methods

Lentil lectin was purchased from Pharmacia Fine Chemicals.

2.1. *Isolation of LATH lectin and its subunits*

The seeds of *Lathyrus odoratus* were purchased from Norwegian Seeds A/S. The material precipitated at 30–60% ammonium sulphate was chromatographed on a column with DEAE–Sepharose CL-6B as in [1]. The proteins not retained on the column (fraction no. I) [1] were dialyzed against 0.015 M phosphate buffer containing 0.15 N NaCl (pH 7.4) and filtered through a column of Sephadex G-100 in the same buffer. The LATH lectin adsorbed to the column was eluted with 0.1 M glucose in the phosphate buffer.

The subunits of the LATH lectin were isolated by gel filtration on a Sephadex G-100 column equilibrated with 2 M acetic acid or with 6 M guanidine–HCl in 1 M acetic acid.

2.2. *SDS–polyacrylamide gel electrophoresis*

The electrophoresis was performed in a Laemmli [5] system as in [1].

2.3. *Metal analysis*

The content of manganese and calcium in the LATH lectin was determined by atomic absorption.

2.4. *Ultracentrifugation*

The sedimentation equilibrium experiments were carried out in a Beckman Analytical Ultracentrifuge, model E at 21°C for the whole lectin and the heavy subunit and at 4°C for the light subunit. The whole lectin molecules were analyzed in the above phosphate buffer while the isolated chains were dissolved in 6 M guanidine–HCl in 0.1 M Tris–HCl (pH 8.0).

2.5. *Protein determination*

The protein concentration was measured by the method in [6] with bovine serum albumin as standard.

2.6. *Amino acid analysis and sequence determinations*

Protein samples were hydrolyzed under reduced

Abbreviations: Con-A, *Canavalia ensiformis*; favin, *Vicia faba*; LATH, *Lathyrus odoratus*; lentil, *Lens culinaris*; pea, *Pisum sativum*; *V. cracca*-Glc, *Vicia cracca*-glucose specific lectin

pressure in 6 M HCl for 16 h, and the amino acid analysis done as in [7]. A Jeol-JAS-47K sequence analyzer was used for automated Edman degradation. Reagents and solvents used were obtained from Pierce Chemical Co. The phenylthiohydantion (PTH) amino acids were analysed by thin-layer chromatography (TLC), by gas chromatography and amino acid analysis of hydrolysates of the PTH-amino acids [7,8]. In addition to these identification methods a TLC system utilizing Merck DC-Alufolien Kieselgel 60 F 254 art. 5544 in the solvent chloroform/ethanol/methanol (88.2:1.8:10, v/v/v) was used.

3. Results and discussion

We have now purified the LATH lectin by affinity chromatography on Sephadex G-100. Like the LATH preparation isolated by conventional chromatography [1] it showed two bands upon SDS gel electrophoresis. Similar bands were obtained for the lentil lectin run in parallel (not shown). Since this lectin is known to be composed of two light chains (α) and two heavy chains (β) it indicates that the LATH lectin has a similar subunit structure. In keeping with this nomenclature and based on the sequence data below, we have designated the LATH subunits as α and β.

The LATH chains were separated on Sephadex G-100 in the presence of 2 M acetic acid. The 1 M acetic acid used in [9] to separate the lentil subunits, resulted in no separation of the LATH chains. The individual chains, as well as a molar mixture of the chains, were stepwise dialyzed back to neutral conditions and tested for hemagglutination which showed negative results. A similar loss of activity has been reported for the lentil lectin also [9]. Addition of

Table 1
Amino acid composition of the LATH lectin and the isolated subunits[a]

Amino acid	Whole LATH	Heavy chain	Light chain
Asp	11	16	4.6
Thr	12	11	9.7
Ser	11	10	14
Glu	9.2	7.5	13
Pro	3.9	3.8	4.3
Gly	7.8	8.0	11
Ala	8.2	7.7	8.4
Val	8.4	7.5	9.6
Ile	3.3	4.0	1.3
Leu	6.8	5.5	8.0
Tyr	3.1	3.7	1.6
Phe	7.1	6.8	5.8
His	1.5	0.92	3.7
Lys	5.3	5.7	4.0
Arg	1.3	1.1	1.5

[a] Expressed as residue/100 residues

Table 2

A comparison of the N-amino terminal sequences of lectins with heavy and light chains

Light chains (α)

	1	5	10	15	20	25
LATH (this work)	Val Thr Ser Tyr Thr Leu Asn Glu Val Val Pro Leu Lys Asp Val Val Pro Glu Trp Val Arg Ile Gly Phe Ser					
Lentil (ref.12)	———————————					
V.c. Glc (ref.13)	———————————	Ser Asp	———————————			
Pea (ref.14)	———————————	Ser Asp	——— Ser	———————————		(?)
Favin (ref.11)	Leu —— Gly	——— Ser	———————————			

Heavy chains (β)

	1	5	10	15	20	25
LATH (this work)	Thr Glu Thr Thr Ser Phe Leu Ile Thr Lys Phe Ser Pro Asp Gln Gln Asn Leu Ile Phe Gln Gly Asp Gly Tyr					
Lentil (ref. 12)	———————————	Ser	———————————			
V.c. Glc (ref.13)	———————————					
Pea (ref.3)	———————————					Asn ———

	1	5	10	15	20	26
Favin (ref.15)	Thr Asp —	Ile ———	Ser —— Pro ———	Arg ———	Pro ———————	Gly ———

1 mM $CaCl_2$ and $MgCl_2$ during dialysis did not restore the activity. Metal analysis showed that the LATH lectin contained 0.14 ± 0.12% Mn and 0.13 ± 0.053% Ca. The lentil lectin contains 0.21% Mn and 0.15% Ca [9].

By sedimentation equilibrium ultracentrifugation the M_r of the whole LATH lectin was found to be 40 000 while the subunits were M_r 17 000 and 4400, respectively. On the basis of these data a stoichiometry of $(\alpha\beta)_2$ would give M_r 42 800 for the whole lectin.

Table 1 shows the amino acid composition of the lectin and its subunits. The LATH lectin contains neither methionine nor cysteine. This has been reported for other glucose–mannose binding lectins such as pea [10] and favin [11]. The amino acid analysis revealed no hexosamines.

The N-terminal sequences of residues 1–25 of the LATH subunits, determined by automated Edman degradation were compared with the sequences of other glucose–mannose-binding lectins (table 2). Extensive homologies with the lentil [12], *V. cracca*-Glc [13] and pea lectin [3,14] were seen (table 2). There was also an extensive sequence homology with the α chain of favin [11], whereas the β chain of favin [15] shows only limited homology. The favin β subunit was compared with the other chains from amino acid residues in positions 2–26.

According to normal taxonomy, *Canavalia* belongs to the *Phaseoleae* tribe, whereas *Lathyrus* as well as *Lens*, *Vicia* and *Pisum* belong to the *Vicieae* tribe of the *Leguminoseae* family. The close relationship between *Lathyrus odoratus* and other species in the *Vicieae* tribe has been confirmed here by similarities in subunit structure and N-terminal sequence of their lectins.

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References

[1] Kolberg, J. (1978) Acta Pathol. Microbiol. Scand. C86, 99–104.
[2] Kolberg, J. and Michaelsen, T. E. (1979) Acta Pathol. Microbiol. Scand. C87, 275–279.
[3] Foriers, A., Wuilmart, C., Sharon, N. and Strosberg, A. D. (1977) Biochem. Biophys. Res. Commun. 75, 980–986.
[4] Reeke, G. N., Becker, J. W., Cunningham, B. A., Gunther, G. R., Wang, J. L. and Edelman, G. M. (1974) Ann. NY Acad. Sci. 234, 369–382.
[5] Laemli, U. K. (1970) Nature 227, 680–685.
[6] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[7] Sletten, K. and Husby, G. (1974) Eur. J. Biol. Chem. 41, 117–125.
[8] Pisano, J. J. and Bromzert, T. J. (1969) J. Biol. Chem. 244, 5597–5607.
[9] Fliegerová, O., Salvetová, A., Tichá, M. and Kocourek, J. (1974) Biochim. Biophys Acta 351, 416–426.
[10] Mařík, T., Entlicher, G. and Kocourek, J. (1974) Biochim. Biophys. Acta 336, 53–61.
[11] Hemperly, J. J., Hopp, T. P., Becker, J. W. and Cunningham, B. A. (1979) J. Biol. Chem. 254, 6803–6810.
[12] Foriers, A., De Neve, R., Kanarek, L. and Strosberg, A. D. (1978) Proc. Natl. Acad. Sci. USA 75, 1136–1139.
[13] Bauman, C., Rüdiger, H. and Strosberg, A. D. (1979) FEBS Lett. 102, 216–218.
[14] Van Driessche, E., Foriers, A., Strosberg, A. D. and Kanarek, L. (1976) FEBS Lett. 71, 220–222.
[15] Cunningham, B. A., Hemperly, J. J., Hopp, T. P. and Edelman, G. M. (1979) Proc. Natl. Acad. Sci. USA 76, 3218–3222.