The amino acid sequences of the $\alpha 1$ and $\alpha 2$ subunits of the isoelectins from seeds of *Lathyrus ochrus* (L) DC

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The complete amino acid sequences of the α1 and α2 subunits of the isolectins (LoL I and LoL II) from seeds of *Lathyrus ochrus* were determined by analysis of peptides derived from the proteins by digestion with trypsin, chymotrypsin and the *S. aureus* V8 protease. Both subunits consisted of single polypeptide chains of 53 amino acids, which differed from one another in only 4 positions near their C-termini, and exhibited high homology to the light (α) chains of the lectins from *Lathyrus sativus, L. odoratus* and a number of other legume seeds.

Lathyrus ochrus *isoelectin* *α Subunit* *Amino acid sequence* *Homology* *Legume lectin*

1. INTRODUCTION

Seeds of various legume species belonging to the Vicieae tribe have been shown to contain D-glucose- and D-mannose-specific mitogenic lectins composed of two heavy (β) chains ($M_r \sim 17\,000$) and two light (α) chains ($M_r \sim 6000$ [1-4]. A similar lectin was recently isolated from seeds of *Lathyrus ochrus* which could be separated into two isolectin forms (LoL I, p*I* 7.2; LoL II, p*I* 6.0) by chromatofocusing in the pH range 8.4–5.0 [5]. Gel filtration of the LoL I and LoL II isolectins in the presence of 6 M guanidine HCl yielded, in both cases, two peaks corresponding to the molecular masses of heavy (β) chains (17 kDa) and light (α) chains (6 kDa) which supported the hypothesis that the two related isolectins had ($\alpha 1\beta 1$)2 and ($\alpha 2\beta 2$)2 structures respectively [5]. We now report the complete amino acid sequences of the two light ($\alpha 1$ and $\alpha 2$) subunits and their homology to the α chains from other legume lectins.

2. MATERIALS AND METHODS

2.1. *Materials*

Seeds of *L. ochrus* (L.) DC were obtained from plants cultivated under field conditions.

2.2. *Isolation of* L. ochrus *lectin (LoL)*

The lectin was extracted from a crude seed meal with 50 mM Tris, 150 mM NaCl (pH 7.6) buffer, and subjected to a fractional precipitation with $(NH_4)_2SO_4$. The 30–60% $(NH_4)_2SO_4$ precipitate dissolved in the extraction buffer was dialyzed against the same buffer and applied to a Sephadex G-100 column equilibrated with the same buffer. The *L. ochrus* lectin retained by the column was eluted by adding 0.1 M glucose to the eluting buffer, precipitated with 90% (w/v) $(NH_4)_2SO_4$ and extensively dialyzed against the Tris-buffered saline.

2.3. *Separation of LoL I and LoL II isolectins*

The *L. ochrus* lectin (50 mg) was applied to a PBE 94 (Pharmacia AB) chromatofocusing column (1 × 30 cm) equilibrated with 25 mM Tris–acetic acid (pH 8.4). The elution was performed with a 10-fold diluted mixture of Polybuffer 96 (30%, v/v) and Polybuffer 74 (70%, v/v Pharmacia AB) adjusted to pH 5.0 with 1 M acetic acid. Two main peaks were obtained corresponding respectively to LoL I (p*I* 7.2) and LoL II (p*I*

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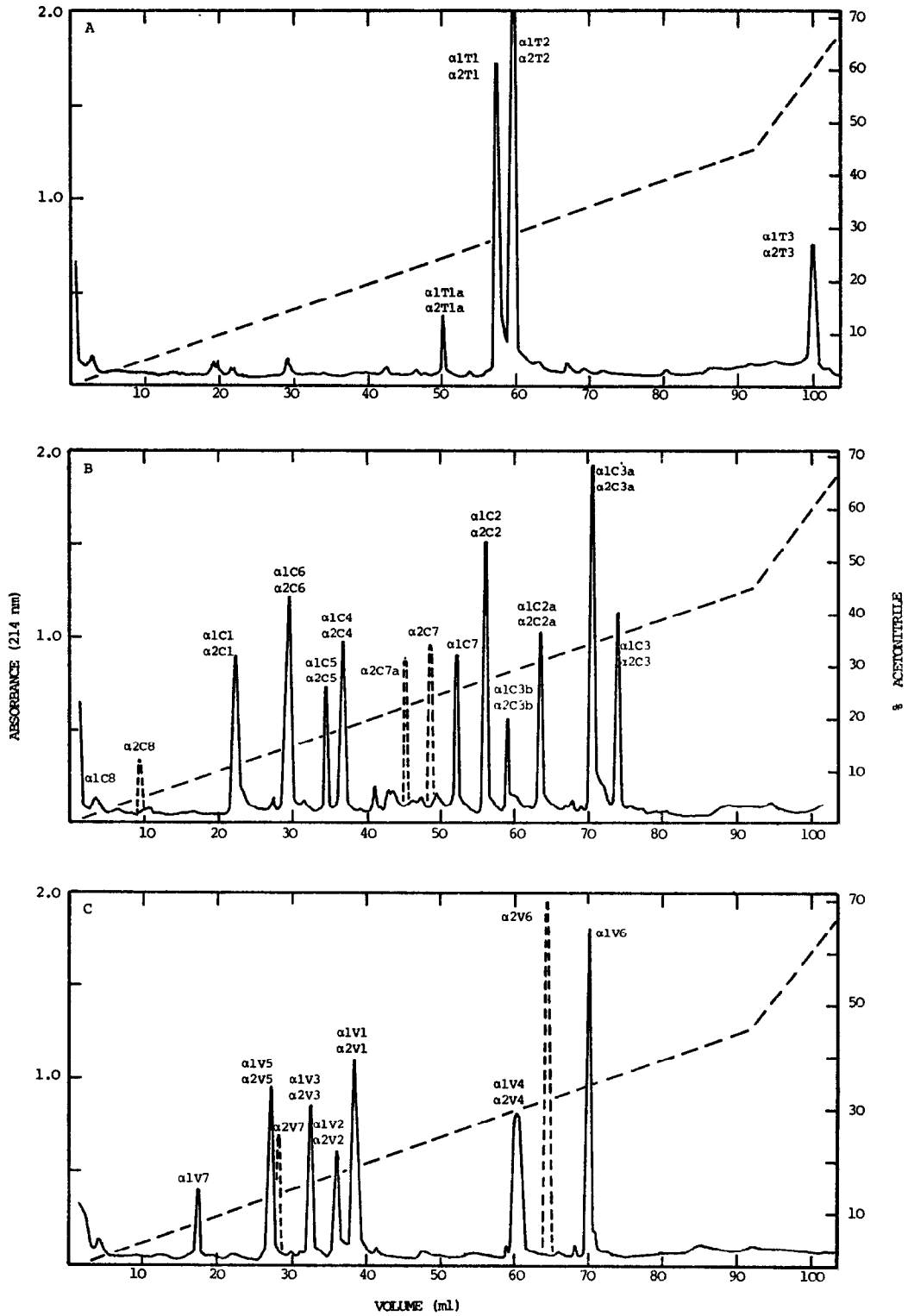

Fig. 1. See following page for figure legend.

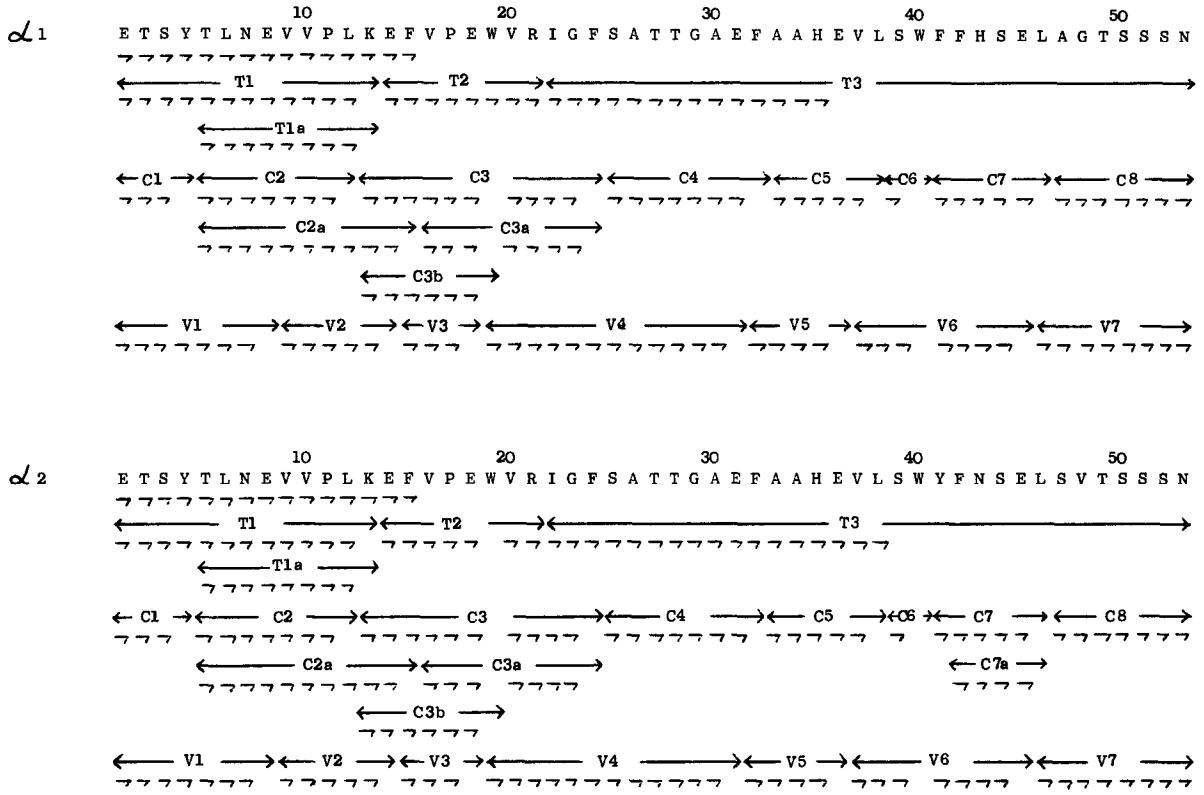

Fig. 2. The amino acid sequences for the α1 and α2 subunits of the isolectins from seeds of *Lathyrus ochrus* (L.) DC. T, tryptic peptides; C, chymotryptic peptides; V, peptides from digestion with *S. aureus* V8 protease. (¬) Amino acids identified by DABITC micro sequencing method.

6.0). The isolectins were precipitated with 90% (w/v) $(NH_4)_2SO_4$ and extensively dialyzed against distilled water.

2.4. *Separation of isolectin subunits*

The α and β subunits of the *L. ochrus* isolectins were separated by gel filtration on a column of Biogel P-60 equilibrated with 6 M guanidine HCl. Two peaks corresponding respectively to the heavy β (first peak) and light α (second peak) subunits were obtained for both LoL I and LoL II. The guanidine HCl was eliminated by extensive dialysis against distilled water and the precipitated subunits lyophilized.

2.5. *Enzyme digestions and separation of peptides*

Samples (2,5 mg) of the α subunits were digested separately with trypsin, chymotrypsin and *S. aureus* V8 protease as in [6]. Mixtures of peptides produced by these methods were purified by reverse-phase high performance liquid chromatography (HPLC) on a Micropac MCH-10 column (0.4×30 cm, Varian) in a Varian model 5000 HPLC apparatus using a linear gradient of 0–70% acetonitrile (HPLC grade S, Rathburn, Peebles) in 0.1% trifluoroacetic acid. Peptides were detected by measuring the absorbance at 214 nm.

Fig. 1. Reverse-phase HPLC separation of (A) tryptic, (B) chymotryptic and (C) *S. aureus* V8 peptides derived from the α1 and α2 subunits of the isolectins from *L. ochrus*. The separations were achieved on a Micropac MCH-10 column (0.4×30 cm) using a linear gradient of 0–70% acetonitrile (– – –) in 0.1% trifluoroacetic acid. Solid lines indicate absorbance at 214 nm for α1 peptides and for α2 peptides which had the identical elution times. Dashed lines indicate positions of α peptides which differed in elution time from corresponding α1 peptides.

Table 1

The amino acid compositions of the *Lathyrus ochrus* isolectin subunits α1 and α2

Amino acid	LoL I (α1 subunit)		LoL II (α2 subunit)	
	Analysis	Sequence	Analysis	Sequence
ASP	2.12	2	2.91	3
THR	4.93	5	5.09	5
SER	5.46	7	7.05	8
GLU	4.93	7	5.83	7
PRO	2.60	2	2.12	2
GLY	3.92	3	2.33	2
ALA	6.04	5	4.40	4
VAL	5.83	5	7.42	6
ILE	1.32	1	1.12	1
LEU	3.82	4	4.45	4
TYR	0.74	1	1.27	2
PHE	5.67	5	4.82	4
LYS	1.70	1	1.38	1
HIS	2.07	2	1.11	1
ARG	1.22	1	1.01	1
TRP	ND	2	ND	2

Residues expressed as mol per mol protein. ND, not determined. No corrections were made for decomposition or incomplete hydrolysis.

2.6. *Sequence determination*

The intact α subunits and the peptides derived from them by the various enzymic digestions were subjected to micro-sequence analysis using the 4-*N*,*N*-dimethylaminoazobenzene-4′-isothiocyanate (DABITC)/phenylisothiocyanate (PITC) double coupling method [7].

2.7. *Amino acid analysis*

The amino acid compositions of the α subunits were determined on a Beckman *L. ochrus* lectin and the isolectins LoL I and LoL II cross-reacted in double diffusion tests [13] with lectins from other *Lathyrus* spp. and with lectins from other members of the tribe Vicieae (pea, lentil, broad bean) but not with Con A or the soybean lectin [5].

The purities of subunits α1 and α2 were confirmed by PAGE at basic and acidic pH, by isoelectric focusing and by N-terminal analysis by the dansyl chloride and by the DABITC/PIIC double coupling methods. The tryptic peptides of both α1 and α2 gave rise to identical profiles when they were separated by reverse-phase HPLC (fig. 1a). On the other hand, the reverse-phase HPLC profiles yielded by the chymotryptic (fig. 1b) and *S. aureus* V8 peptides (fig. 1c) showed small but distinct differences between subunits α1 and α2, which were due to the amino acid differences near their respective C-termini.

The complete amino acid sequences of subunits α1 and α2 are shown in fig. 2 together with the details of the peptides from which they were deduced. Both proteins consisted of 53 amino acids, and only differed in the amino acids found

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                   10                  20                  30                  40                  50
a)  E T S Y T L N E V  V P L K E F V P E W  V R I G F S A T T G  A E F A A H E V L S  W F F H S E L A G T  S S S N
b)  - - - - - - - - -  - - - - - - - - - -  - - - - - - - - - -  - - - - - - - - - -  - Y - N - - - S V -  - - - -
c)  V - - - - - - - -  - - - - D V - - - -  - - - - - - - - - -  - - - - - - - - - -  - S - - - - - G - -  - G - Q K
d)  V - - - - - - - -  - - - - D V - - - -  - - - - - - - - - -  - - - - - - - - - -  - S - - - - - D/G - -  - A - K Q S
e)  V - - - - - S D -  - S - - D V - - - -  - - - - - - - - - -  - - Y - - - - - - -  - S - - - - - S - -  - - K Q
f)  L - G - - - S - -  - - - - D V - - - -  - - - - - - - - - -  - - Y - T - - - - -  - T - L - - - T - P  - N
g)  S - - - G - S A -  - - - - D V - - - -  - - - - - - - - - -  D - Y - - Q - - H -  - S - - - - - G - -  - -
h)  V - - - - - S D -  - - - - D V - - - -  - - - - - - - - P -  - - Y - - - - - - -  - S - - - - - S - -  - - K Q
i)  V - - - - - - - -  - - - - D V - - - -  - - - - - - - - - -  - - - - - - - - - -  - S - N - Q - G H -  - K -
   70                  80                  90                  100                                   122
j)  A D A T S V S Y D  - D - N D V L - - -  - - V - L - - S - -  L Y K E T N T I - -  - S - T - K - K S N  - T H Q
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Fig. 3. Homology of the amino acid sequences of the α1(a) and α2(b) subunits of the *L. ochrus* isolectins with the α chains of the lectins from (c) *L. odoratus* [3]; (d), *L. sativus* [4]; (e) *Pisum sativum* [2]; (f) *Vicia faba* [1]; (g) *V. Sativa* [14]; (h) *V. cracca* [15]; (i) *Lens culinaris* [16] and (j) residues 70–122 of Con. A [17]. (——) Same amino acid as in *L. ochrus* α1 sequence.

in positions 41, 43, 47 and 48 near their C-termini. The M_r values calculated from the amino acid sequences were 5878 and 5928 for subunits $\alpha 1$ and $\alpha 2$, respectively, which are in good agreement with the estimates of the sizes of the subunits made by SDS-PAGE. The sequences shown in fig. 2 are also in agreement with the amino acid compositions of the subunits (table 1) except for the small discrepancies in the values for serine, glutamic acid and valine.

Fig. 3 shows a comparison of the amino acid sequences of the *L. ochrus* $\alpha 1$ and $\alpha 2$ subunits with the sequences of the light (α) chains from a number of other legume lectins. As might be expected the *L. ochrus* α subunits showed the greatest resemblance to the α chains of the other *Lathyrus* species which have been sequenced [3, 4] differing only in positions 1, 14, 15, 41, 43 (*L. ochrus* $\alpha 2$), 47, 51 and 53. There was also strong homology with the light (α) chains of the model 119 BL amino acid analyzer. The compositions of the peptides were determined in a Varian 5000 HPLC fitted with a Micropak hydrolysate amino acid column in the sodium form and using a post-column *o*-phthalaldehyde reactor system with a Fluorichrom detector as described in [6]. The presence of tryptophan in peptides was detected by staining on paper with *p*-dimethylaminobenzaldehyde.

3. RESULTS AND DISCUSSION

The yield of the *L. ochrus* lectin after affinity chromatography was approx. 600 mg/kg of seed flour [5]. The lectin exhibited two distinct bands on polyacrylamide gel electrophoresis (PAGE) at basic pH [8] and a single diffuse band at acidic pH [9]. Measurement of the A_{280} of unfixed gels after PAGE at basic pH in quarts tubes indicated that the proportions of the two isolectins LoL I and LoL II were 36 and 64%, respectively. Atomic absorption revealed that the lectin contained 2.2 Mn^{2+} and 2.7 Ca^{2+} atoms per molecule, on the basis of an M_r of 49 000 estimated by gel filtration on a column of AcA 54 in 0.1 M phosphate-buffered saline at pH 7.2 [5]. The carbohydrate content of the lectin determined as in [10] using D-glucose as standard, was less than 0.25% by weight and no amino sugars were detected [5].

Analytical isoelectric focusing [11] of the lectin gave two distinct fractions, LoL I and LoL II, whose isoelectric points were respectively pH 7.2 and pH 6.0. After these two isolectins were separated by chromatofocusing they each gave only a single band by PAGE at basic pH, but SDS-PAGE [12] showed that both isolectins consisted of two subunits whose apparent molecular masses were estimated to be 4.5 kDa and 20 kDa for the light (α) and heavy (β) subunits, respectively. Isoelectric focusing between pH 8.0 and 5.0 in PAGE rods containing 8.0 M urea also confirmed only one species of α and one species of β subunit for each isolectin [5].

Both LoL I and LoL II agglutinated equally well human erythrocytes of different ABO groups. Their hemagglutinating activity was inhibited best by D-mannose, D-glucose and their methyl α-glycosides [5]. Antibodies raised in rabbits against the lectins from the genera *Pisum* [2], *Vicia* [1,14,15] and *Lens* [16], all of which belong to the Vicieae tribe of the family Leguminosae, but much less homology with the region 70–122 of the single-chain lectin Con A [17]. A notable feature of both *L. ochrus* $\alpha 1$ and $\alpha 2$ is that they possessed in positions 14 and 15, instead of aspartic and valine which are invariant in these positions in all of the comparable lectins.

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