A COMPARISON OF THE TWO LECTINS FROM *VICIA CRACCA*

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1. Introduction

Structural studies of lectins purified from leguminous plants have revealed the existence of two homologous groups of proteins:
(1) Composed of a single polypeptide chain;
(2) Composed of a small α and a large β chain.
We have postulated that the extensive sequence similarities suggest a common origin for these two types of lectins [1]. The data could be interpreted to mean that both types of proteins derived from each other by proteolytic cleavage. Alternately we proposed that they were both products of homologous genes which diverged during evolution. We describe here for the first time the simultaneous presence of the two types of lectins in *Vicia cracca.* Our data confirm the existence of at least two genes coding for lectins in a single plant. These genes probably originated from each other by duplication.

2. Materials and methods

Vicia cracca, subspecies *cracca,* seeds were collected near the University of Würzburg. The blood group A-specific lectins were extracted using affinity chromatography as in [2].

The glucose-specific lectin was purified from the *Vicia cracca* extract by adsorption on Sephadex G-100. Elution of this protein was by buffer containing 0.1 M glucose [3]. The two subunits were separated on a Biogel P 60 column (2 × 100 cm) in 1% SDS. Prior to the separation, the lectin was treated in SDS at 60°C for 30 min. Polyacrylamide gel electrophoresis in the presence of SDS was carried out by the method in [4]. The gels were stained for protein with Coomassie brilliant blue R.

Sequence analysis was performed with automated Edman degradation on 0.05–0.1 μmol of purified subunits with a Beckman 850 C sequencer using 0.33 M Quadrol and single acid cleavage [5]. Phenylthiohydantoin amino acids were identified by gas–liquid chromatography [6], thin-layer chromatography [7] and high performance liquid chromatography [8]. Antibodies were raised against the *Vicia cracca* A-specific lectin and against the glucose-specific protein in rabbits using complete Freund adjuvant. Immunodiffusion experiments were performed in agar plates using 3% polyethyleneglycol to improve the precipitin reaction.

3. Results and discussion

The glucose-specific lectin which agglutinates erythrocytes unspecifically was found in ~0.15% (w/w) of the *Vicia cracca* seeds. The lectin was purified by affinity chromatography on the glucose–polymer Sephadex G-100. SDS electrophoresis showed two sharp bands corresponding to mol. wt 7000 and 21 000. The lectin contains ~4% *N*-acetylgalactosamine. The two subunits could not be disso-

Abbreviations: Con A, concanavalin A; PHA, phytohemagglutinin from *Phaseolus vulgaris;* PNA, peanut agglutinin; SBA, soybean agglutinin; *V. cracca*-GalNAc, *Vicia cracca N*-acetyl-D-galactosamine specific lectin; *V. cracca*-Glc, *Vicia cracca*-glucose specific lectin

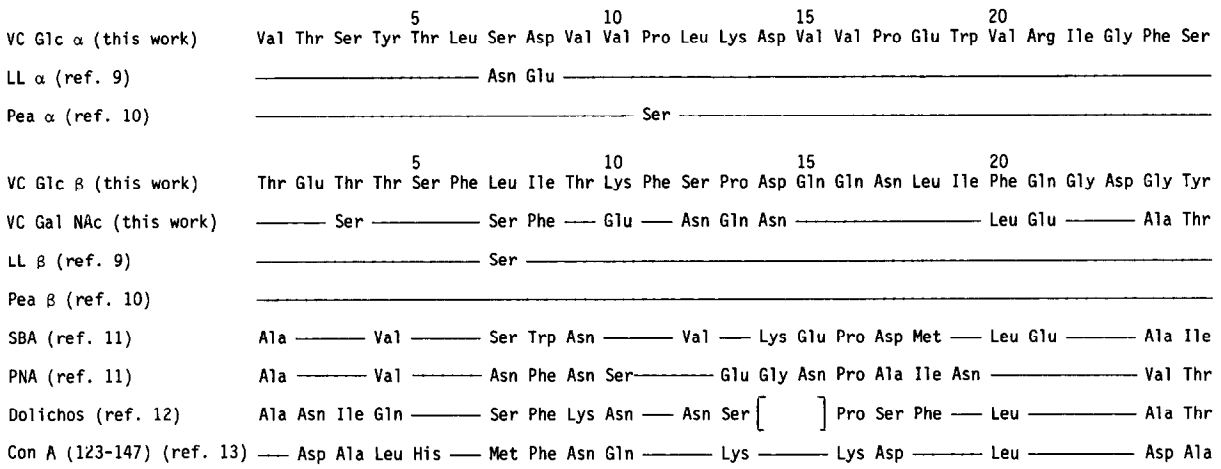

Fig.1. A comparison of one chain and two chain lectin amino-terminal sequences.

ciated in 6 M guanidine–HCl. In 1% SDS, there was a separation of ~20% without and of almost 100% after heating. Automated Edman degradation was performed on each chain (fig.1).

The two isolectins of the blood group A-specific lectin [2] were separated using affinity chromatography and elution at different pH values. Their amino terminal sequences were identical, however and similar to other one chain lectins as SBA, PNA, *Dolichos* and Con A.

The comparison of the sequences reveals extensive homologies between the N-terminal regions of the α- and β-chains of *Vicia cracca* glucose-specific lectin and the corresponding chains of the lentil [9] and pea lectins [10,12]. For instance, the β-chain of the glucose-specific *Vicia cracca* lectin is identical to the pea chain and differs from the corresponding lentil sequence at only one position (fig.1). On the other hand, there is no homology between the α- and the β-chain of the unspecific lectin and only limited homology between the β-chain of the unspecific and the A-specific lectin. Thus, the unspecific lectin from *Vicia cracca* bears more resemblance to the lectins from pea and lentil than to the A-specific one from the same plant.

The amino terminal sequence determinations indicate that the two lectins from *Vicia cracca* do not originate one from each other since sequence differences in homologous regions are shown. Instead, our data suggest that these two lectins are coded for by two genes, which have a common origin, but which diverged a number of years ago.

The divergence reveals itself not only in sequence differences, but also by different sugar specificities; the agglutination of erythrocytes from blood group A with the A-specific lectin is inhibited by galactose or *N*-acetylgalactosamine whereas the agglutination of blood group O erythrocytes is inhibited by glucose.

These divergences were confirmed in immunochemical studies: there is no crossreaction between antibodies against the O-specific lectin and the A-specific lectin. These studies also confirm the sequence homologies: antibodies against the lentil lectin crossreact with the glucose-specific lectin but not with the A-specific lectin.

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