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Short communication

Subunit composition of proteins from seeds of Lupinus albus

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Abstract. All the main globulins in the seeds of *Lupinus Albus* are oligomeric glycoproteins. Legumins (33%) consist of two similar protein molecules which contain protomers linked by disulphide bridges. They result from a partial proteolytic breakdown of an original polypeptide chain. Vicilins (44%) consist of four similar protein molecules with several protomers linked together by non-covalent bonds. Globulin 1 (6%) has a native M.W. of 199 kd and is formed by four 45.0 kd subunits consisting of two smaller protomers (28.0 and 16.0 kd) linked by –S–S– bonds. Globulin 9b (12.5%) has the lowest M.W. (44.0 kd) and is made up of three protomers, two of which are linked by disulphide bonds.

Albumins, 12% of the total seed proteins of *Lupinus albus,* contain a variety of species. Globulins, which comprise the remaining protein (88%), can be separated into 12 components. Of these 6 represent 91% of the total globulins. They were isolated and purified as described in ref [1]. Various aspects of their molecular structure were studied [1, 2]. The residual minor components were not further investigated.

Two major groups, the vicilins and the legumins, can be identified; proteins in each group are highly similar as indicated by their aminoacid composition and behaviour on the ion exchanger, the amount and type of bound carbohydrate present and the type and number of constituent protomers observed.

All globulins have high molecular weights (M.W.), except protein 9b which has a M.W. of 44.0 kd. They all contain more than one protomer. In some of them, like the legumins and the globulin 1 and 9b, protomers are bound by interpeptide –S–S– bridges. All globulins studied are glycoproteins: bound carbohydrate varies from 0.7% to 4.3% depending on the protein considered [1].

Legumins (proteins 8 and 9a) represent 33% of the total globulins and differ from one another because of different bound sugar contents and ionization properties which are probably related to the degree of amidation of Asp and Glu residues. The two legumins differ also in type and amount of peptides observed by subunit analysis. Indeed globulin 8 displays three major subunits of M.W. 64.0, 55.5 and 44.0 kd which all contain two

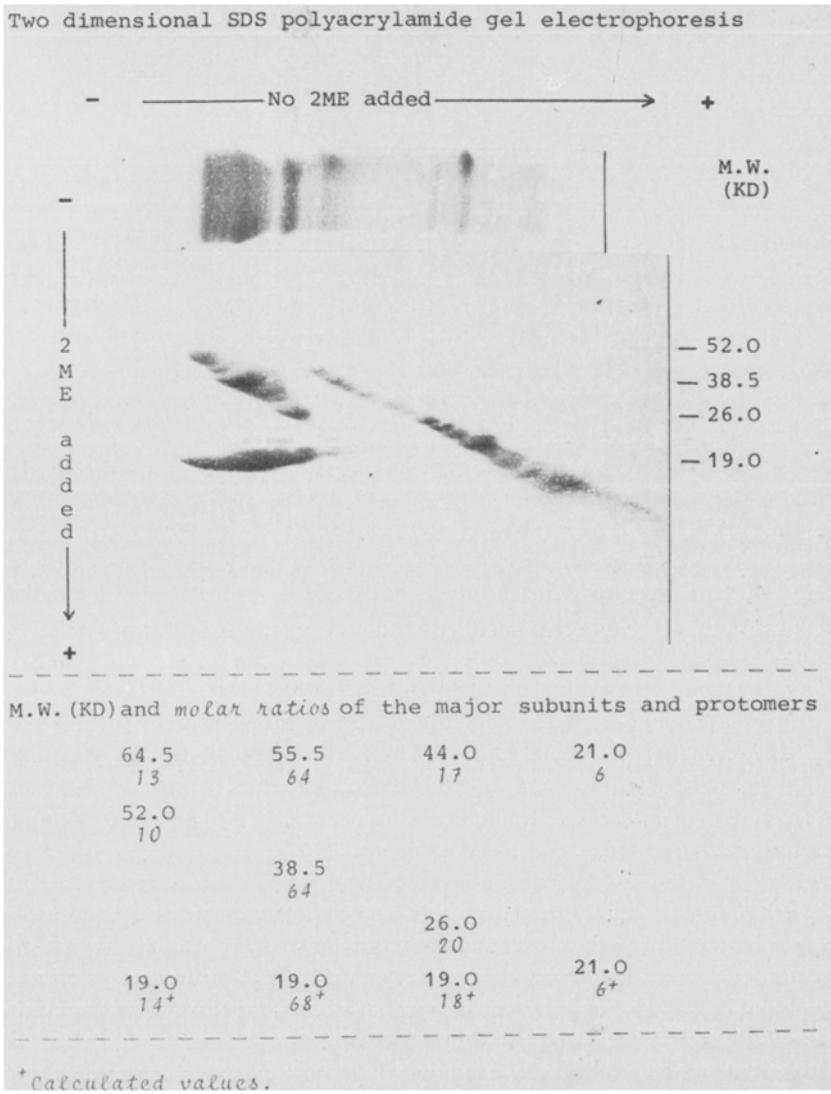

Figure 1. Subunit and protomer composition of globulin 8.

protomers linked by an interpeptide disulfide bond, one of which in all cases has a M.W. of 19.0 kd. Moreover the protein has one single peptide protomer of 21.0 kd which does not undergo reduction. The SDS-PAGE pattern and molecular ratios of these different components are shown in Figure 1.

Considering that all the molecules in globulin 8 have the same M.W. and amino acid composition, the characteristics mentioned do not allow us to establish a molecular composition in which all the determined peptides are represented as individual subunits. To account for the variety of isolated

Table 1. Subunit and protomer composition of globulins 1 and 9b

Globulin		Subunits and (S–S bound protomers) in the protein		
	M.W. (kd)	M.W. (kd)		number
1	199	(28 + 16)	x	4
9b	44	(11 + 11) + 13		1

components and the homogeneity of the molecule we suggest that an original peptide of M.W. 64.5 kd has been proteolytically nicked to a large extent into fragments of M.W. (38.5 + 19.0) + 9.0, not seen in SDS-PAGE, and 21.0 + (26.0 + 19.0). Peptides within parenthesis are those linked by –S–S– bonds. All these protomers remain together in the seed to yield the 64.5 kd subunit of the molecule, but are liberated and separately determined by denaturation of the protein.

For globulin 9a a similar process is suggested for an original peptide of apparent M.W. of 61.5 kd which yields on fragmentation protomers of (36.5 + 19.0) + 6.0, not seen in SDS-PAGE, 21.0 + (17.5 + 19.0) + 4.0, not seen in SDS-PAGE and 16.5 + (25.5 + 19.0), the most conspicuous of which is the first group.

Close to the components mentioned, densitometric scanning of the electrophoretic gels reveals small amounts of other components with similar but not identical M.W. (SDS-PAGE) and/or pI (IEF). This indicates microheterogeneity at the level of subunits which may depend on a different extent of amidation of Glu and Asp and on the presence of carbohydrate mainly in the highest M.W. subunits.

Vicilins (proteins 4, 5, 6, 7) represent 44% of the total globulins and do not display interpeptide –S–S– bonds. Their subunit composition is qualitatively identical and quantitatively very similar. The number of protomers is high, which makes it difficult to trace the origin of the different peptides as was done for the legumins. However, the pattern found likely depends on a situation similar to that described for the legumins. The molecular individuality of vicilins has to be attributed to different sugar content and/or to a different number of subunits in each molecule.

Globulin 1, M.W. 199 kd, is made up of four subunits of 45.0 kd, each formed by two peptides of M.W. respectively of 16.0 and 28.0 kd, linked by –S–S– bridges (Table 1). Only the heavier protomer contains bound sugar (8.6% of the protomer in weight). It is less acidic than the light protomer. Charge heterogeneity is evidenced in protomers of the same M.W. by two dimensional IEF/SDS-PAGE under reducing conditions [2] but no M.W. heterogeneity appears.

Globulin 9b (M.W. 44.0 kd) is formed by two protomers respectively of M.W. 22.0 and 13.0 kd. By adding 2-mercaptoethanol (2-ME) the heavier one is reduced into two equal 11.0 kd protomers (Table 1). This protein

displays the most acidic character among globulins, which is probably due to the unusually high content of Glu (41% of total aminoacids).

Heterogeneity at the subunit level is observed also in the globulins extracted from selected cultivars of *Lupinus albus* and from a single seed: it is therefore an intrinsic character of the proteins in the seed which likely depends on post-translational modifications.

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