Composition and structure of the 12 S globulin from rapeseed (Brassica napus L.)

K.D. SCHWENKE, B. RAAB, P. PLIETZ[+], and G. DAMASCHUN[+]

Academy of Sciences of the German Democratic Republic,
Research Centre for Molecular Biology and Medicine,
Central Institute of Nutrition in Potsdam-Rehbrücke and
Central Institute of Molecular Biology in Berlin-Buch[+]

The 12 S globulin represents a main storage protein in the seeds of Brassica species /1, 2/. 21-33 % of the nitrogen in sodium chloride extracts of defatted rapeseed varieties or 18-28 % of the total seed nitrogen corresponds to this protein /1, 3/. The present paper concerns the investigation of chemical and physico-chemical properties and the spatial structure of the 12 S globulin.

The protein, isolated by sodium chloride extraction, and purified by Sephadex G-200 gel filtration and ion-exchange chromatography on DEAE Sephadex A-50 gave a single fraction in the isoelectric focusing (Fig 1). The corrected isoelectric point amounts to 7.2 /4/.

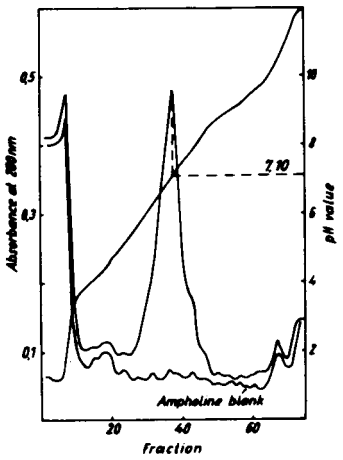

Fig. 1
Isoelectric focusing of the 12 S globulin according to /4/.

The content of characteristic amino acids is shown in Tab. 1. A high amount of aspartic and glutamic acid and a low content of sulphur containing amino acids (half cystine and methionine) are typical. The predominant basic amino acid is arginine. 61 % of the acidic residues (Glu and Asp) are in an amidated form. Calculating the ratio of real acidic (Glu + Asp - Amides) to basic (Σ Lys, His, Arg) residues we obtained a value of 1.0. This ratio points to a relative high basidity and is a further hint to the high isoelectric point of the protein.

The average hydrophobicity $H\phi_{av}$ calculated from the amino acid composition according to BIGELOW amounts to 1041 cal/res (4.36 kJ/res). The corresponding values of a number of other 11/12 S proteins (soybean, hempseed, sunflower and pumpkin seed) are in the range of 944 - 980 cal/res (3.96 - 4.10 kJ/res) /4/.

Tab. 1

Content of characteristic amino acids in the 12 S globulin (residues per 300 000 g protein)

Met	44	
Cys	31	13 SS-bridges
Lys	81	
His	46	271 basic residues
Arg	144	
Asp	270	704 → 272 acidic residues
Glu	434	704 → 423 amide residues

ratio of basic to acidic residues = 1,0

One molecule of the protein contains about 12 disulphide bridges situated in the interior. This value corresponds to the number of polypeptide chains supposed to be present in the protein.

The physico-chemical properties of the 12 S globulin are summarized in Table 2. The molecular weight calculated from the sedimentation and diffusion coefficient amounts to be (300 000 ± 10 000) D /5/. This value is similar to the molecular weights of 11/12 S proteins from other plant seeds.

The secondary structure of the protein determined by circular dichroism measurement is characterized by a low content (11 %) of α-helix and a relatively high content of ß-conformation (31 %) and aperiodical structures (58 %) /6/. These data resemble those of other 11/12 S and 7 S plant proteins.

Tab. 2 Hydrodynamic properties and molecular weight of the 12 S globulin

Sedimentation coefficient $s^0_{20,w}$ $x10^{-13}$s)	12,7
Diffusion coefficient $D^0_{20,}$ ($x10^{-7}cm^2s^{-1}$)	3,8
STOKES radius Rs (nm)	
from quasi-elastic light scattering	5,7
from gel chromatography	5,5
Partial specific volume $\bar{v}$ (ml/g)	0,729
Molecular weight	
$M_{s,Rs}$ (from sedimentation and diffusion)	300000 ± 10000
$M_{s,Rs}$ (from sedimentation and gel chromatography)	294000 ± 13000
Frictional ratio f/fo	1,28

The molecular shape and quaternary structure of the protein were determined by small-angle X-ray scattering and electron microscopy (Table 3) /7 - 9/. According to that, the globulin molecule exists as an oblate ellipsoid of revolution with an axial ratio of 0.8. Both methods deliver proofs for a sphere-like shape of the protein molecule.

Tab. 3 Structural parameters of the 12 S globulin derived from small-angle X-ray scattering /7, 8/ and electron microscopy /9/

	X-ray scattering	Electron microscopy
Molecular shape	oblate ellipsoid of revolution	
Axial ratio	0.80	0.81
Outer dimensions [nm]	11.0 × 11.0 × 8.8	11.3 × 11.3 × 9.2*
Solvation [g solvent/g protein]	0.22	0.49
Radius of gyration [nm]	4.1	
Volume [nm^3]	470	
Surface [nm^2]	440	
Maximum dimension [nm]	11	
Quaternary structure	trigonal antiprism	
Number of subunits	6	6
Symmetry	dihedral point group 32 (D_3)	

* Taking into account the error of determination (11.3 ± 1.1 and 9.2 ± 0.9) the results of both methods are in good agreement

The radius of gyration of the globulin was determined by X-ray scattering to be R_s = 4.1 nm from the scattering curve (Fig 2) /8/. A sphere with the same R_s has a radius of R = 5.4 nm in accordance with the calculated Stokes-radii (Tab 2). This agreement is only valid for compact proteins with a sphere-like shape.

The strongest argument for a sphere-like shape of the 12 S globulin molecule is the high number of subsidiary maxima in the wide-angle region (Fig 2) which is typical for spherical structures.

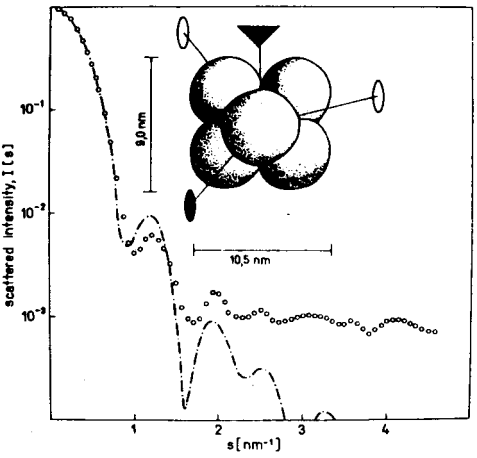

Fig. 2
X-ray scattering curve of the 12 S globulin (ooo) and scattering curve of the quaternary structure model of the protein (-.-.-), according to /8/

The large differences between the frictional ratios f/fo calculated from hydrodynamic parameters (Tab. 2) and from the X-ray scattering curve may be caused by solvation effects. If one assumes a solvation of the globulin molecule of 0.22 g solvent per g protein and a solvation shell of a thickness of 0.5 nm, the ratio of frictional coefficients falls to f/fo = 1.06 which is in much better correspondence with the proposed sphere-like shape.

From the X-ray scattering curve in Fig 2 a model for the quaternary structure of the protein was derived. This consists of a trigonal antiprism built up of 6 subunits with the dihedral point group symmetry 32 (D_3). The calculated excess electron density distribution of the molecule shows that each subunit consists of two domains, which are arranged approximately centro-symmetrically to the centre of gravity of the molecule. A channel (cavity) exists in the centre of the molecule. One domain occupies a smaller volume than the other. The smaller domain is situated nearer to the gravity centre of the molecule.

Like other oligomeric subunits containing proteins, the 12 S rapeseed globulin dissociates in a way depending on milieu conditions. This dissociation reflects the quaternary structure which splits up stepwise. The dissociation is summarized in Fig 3. The 2-3 S component which corresponds to the subunit in the structure model (Fig 2) has a molecular weight of about 50 000 D. Dissociation of the protein by action of sodium dodecyl sulphate/2-mercaptoethanol reveals that the 3 S component obtained after dissociation in the absence of reducing agents is in fact not a real monomeric subunit bit is composed of smaller polypeptide chains bridged by disulphide bonds. The 3 S component therefore represents an intermediary unit.

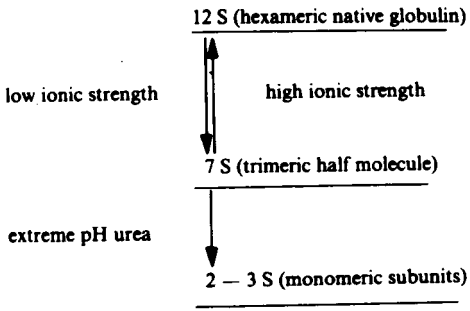

Fig 3
Scheme of dissociation of the 12 S globulin

Fig 4 shows the electrophoresis patterns of the completely denatured and reduced protein. Four protein zones are visible which occur as two double-zones in obviously equal distribution. The following molecular weights were determined: 18 500 ± 800, 21 100 ± 500, 26 800 ± 900, 31 200 ± 1600 D.

Taking into consideration the result of X-ray scattering, we can assume that each of the smaller units combines with one of the larger units to form one "subunit" with a molecular weight of 50 000 D. Therefore a molecular weight of 293 000 would result for the completely associated protein. This is in good agreement with the value of (300 000 ± 10 000)D found by hydrodynamic methods (Fig 1).

In other related 11/12 S proteins the smaller polypeptide chains correspond to basic and the larger ones to acidic chains /10/. Probably, the situation is similar in the rapeseed globulin, but

the proof must be provided by further investigation.

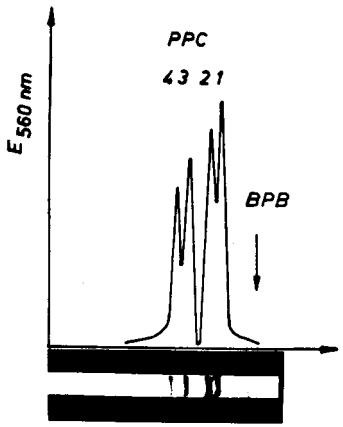

Fig 4
Polypeptide chains patterns of the 12 S globulin after SDS Disc-electrophoresis
PPC 1: M 18 500
PPC 2: M 21 100
PPC 3: M 26 800
PPC 4: M 31 200

The results summarized in this paper shows that the 12 S globulin from rapeseed is characterized by physico-chemical properties and a physical structure which are typical for a great number of 11/12 S proteins in the seed of different plant species and botanical families.

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