The relative molecular mass, heterogeneity and subunit composition of the 12S globulin from oil seed rape

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The 12S globulins represent the major storage proteins in seeds of a variety of plants including legumes. As a group they exhibit considerable homology with respect to size, subunit and amino acid composition, and physicochemical properties (Derbyshire *et al.*, 1976). The principal storage protein of rape seed, although designated a 12S globulin, has had somewhat different reported properties, with a slightly basic isoelectric pH (Schwenke *et al.*, 1981) and different reported M_r values ranging from 129 000 (Gill & Tung, 1976) to 300 000 (Schwenke *et al.*). Work in this laboratory using low-speed sedimentation equilibrium has confirmed the value for the M_r obtained by Schwenke *et al.* (1980) and indirectly supports their proposals concerning the subunit composition of the quarternary molecule. In addition, evidence has been obtained that helps explain the discrepancies regarding the M_r values obtained by different workers.

Crude 12S globulin from rapeseed was isolated according to Wright & Boulter (1974) and purified by chromatography on a Bio-Gel P150 column and then Sepharose CL6B (two passages). The purified 12S globulin was dialysed exhaustively against distilled water and concentrated by means of an Amicon ultrafiltration cell (XM 100A filter). A corrected sedimentation coefficient, $s_{20,w}$, of 11.6×10^{-13} s was obtained from sedimentation velocity measurements (Fig. 1*a*) for a protein concentration of 0.1 mg/ml in a 0.1 M-borate buffer, pH 7.6 containing 0.2 M-NaCl. Only very small amounts of dissociation products of the globulin (2S, 4S and 7S components) were evident, while associated forms were completely absent.

Proteins for sedimentation equilibrium were dialysed in the same buffer for $\gtrsim 12$ h. A Beckman Model E analytical ultracentrifuge was used, equipped with Rayleigh Interference Optics. The methodology for extracting meniscii concentrations, 'whole cell', $M^0_{r,w}$, and point weight average, $M_{r,w}$, relative molecular masses, using the 'intermediate speed method' (Creeth & Harding, 1982) were as described previously (Harding *et al.*, 1987), except that a 30 mm path length cell was used at the lowest possible loading concentration (to minimize possible effects of thermodynamic non-ideality and self-association phenomena). The value used for the partial specific volume was 0.729 ml/g (Schwenke *et al.*, 1980). An $M^0_{r,w}$ of 300 000 $\pm$ 10 000 was obtained by extrapolation of the star point average to the cell base (Creeth & Harding, 1982). This value may be slightly affected by self-association and/or non-ideality. The 'ideal' value (obtained from extrapolation of $M_{r,w}$ to zero concentration (Fig. 1*b*), yielded a value of 280 000 $\pm$ 30 000, which confirms the result of Schwenke *et al.* (1980), who used a less direct procedure (combining sedimentation & diffusion coefficients via the Svedberg equation). It differs significantly from the value obtained by Gill & Tung (1976), who also used a sedimentation equilibrium procedure (but using absorption optics) although their low value may have resulted from anomalous protein adsorption onto the cell windows (Rowe, 1984) or dissociation affects upon freeze-drying. In our own work it was seen that material resuspended in buffer directly after freeze-drying and analysed using sedimentation velocity and equilibrium yielded both dissociation products and aggregates, as also observed by Schwenke *et al.* (1980) for the 12S globulin from sunflower seed, further supporting the homology with other seed proteins (Schwenke, 1975). Our data for the amino acid composition and subunit composition of six subunits (from SDS/polyacrylamide-gel electrophoresis) are also supportive of this homology.

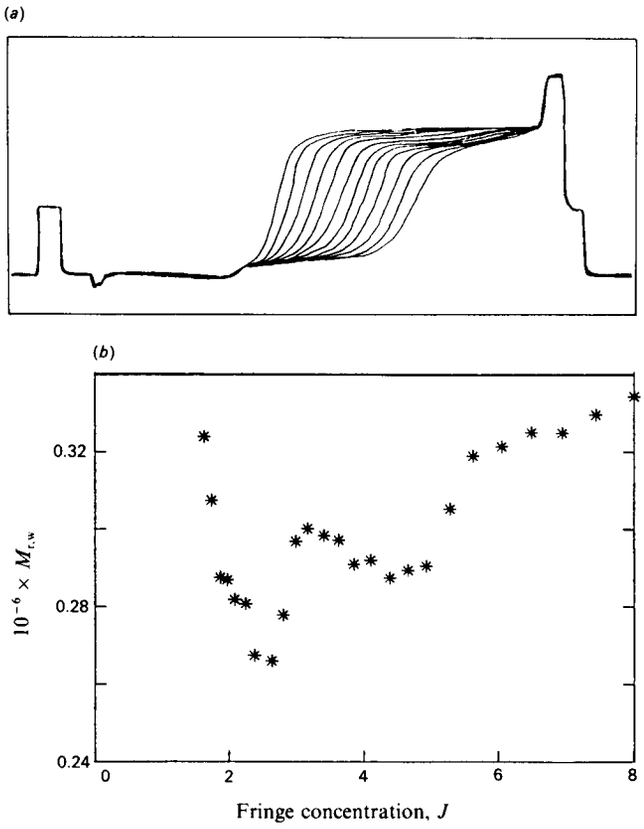

Fig. 1. *Analytical ultracentrifugation of 12S globulin*

(*a*) Sedimentation velocity scans (at 5 min time intervals) of purified 12S globulin from rapeseed. An MSE Centriscan was used, rotor speed 37 000 rev./min, temperature 20.0°C, concentration 0.1 mg/ml. The trace records absorbance at 280 nm (vertcal axis) as a function of radial displacement (horizontal axis). The direction of sedimentation is from left to right. (*b*) Plot of point weight average relative molecular masses from a sedimentation equilibrium experiment in a Beckman Model E analytical ultracentrifuge using Rayleigh Interference Optics. Rotor speed 5227 rev./min, temperature 20.0°C. Initial loading concentration approx. 0.2 mg/ml.

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