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

S. E. HARDING, P. KYLES,
G. WEST and G. NORTON

University of Nottingham, Department of Applied
Biochemistry and Food Science, Sutton Bonington,
LE12 5RD, U.K.

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 quaternary 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 \( \times 10^{-13} \) s was obtained from sedimentation velocity measurements (Fig. 1a) 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 \( \geq 12 \) h. A Beckman Model E analytical ultracentrifuge was used, equipped with Rayleigh Interference Optics. The methodology for extracting menisci concentrations, whole cell, \( M_r \), and point weight average, \( M_{w,r} \), 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 \( M_{r,w} \) 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. 1b), 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.

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