J. Mol. Biol. (1984) **174**, 723–725

LETTERS TO THE EDITOR

Crystallization and Preliminary X-ray Diffraction Studies of Soybean Agglutinin

Soybean agglutinin crystallizes in the monoclinic space group $C2$ with unit cell dimensions $a = 118{\cdot}6$ Å, $b = 88{\cdot}9$ Å, $c = 165{\cdot}9$ Å, $\beta = 103{\cdot}0°$ and one tetramer of 120,000 M_r per asymmetric unit. The crystals are suitable for high-resolution work.

Carbohydrates play a major role in processes involving intercellular recognition, such as the interaction of different cells to form a tissue or adhesion of bacteria to animal cells (Sharon & Lis, 1982). Plant lectins, which are able to recognize and bind specifically to sugars, have been used widely as probes for locating carbohydrate moieties on cell surfaces, and as an aid in clarifying mechanisms of cell–cell recognition (Lis & Sharon, 1973; Roth, 1980).

Soybean agglutinin, specific for *N*-acetyl-D-galactosamine and D-galactose, is one of the best-characterized lectins (Lis *et al.*, 1970; Lotan *et al.*, 1974). It is a tetramer consisting of four subunits of 30,000 M_r each and capable of binding one carbohydrate molecule per subunit (De Boeck *et al.*, 1983). The sugar binding activity of SBA† depends on the presence of Ca^{2+} and a transition metal, which bind to specific sites in each subunit (Jaffe *et al.*, 1977). SBA is a glycoprotein containing four oligosaccharide moieties (Man_9 $(GlcNAc)_2$), one per subunit (Lis & Sharon, 1978). The structure of the carbohydrate chains (Dorland *et al.*, 1981) as well as the amino acid sequence of SBA (Hemperly & Cunningham, 1983) have been determined. When the amino terminus of SBA is aligned with residue 120 of the lectin concanavalin A, the two amino acid sequences show extensive homology, thus suggesting a circular permutation of the sequence (Hemperly & Cunningham, 1983).

We undertook to study the three-dimensional structure of SBA in order to obtain more information on the nature of combining sites of lectins and the conformation of carbohydrate units in glycoproteins. Here we report on the crystallization of SBA.

The lectin was isolated by the method described by Gordon *et al.* (1972), with modifications according to Jaffe *et al.* (1977), and crystallized under the following conditions: 6 mg protein/ml, 0·05 M-MES or HEPES buffer (pH between 6 and 8), 0·01 M-sodium azide and 6% (w/v) polyethylene glycol (6000). Small crystals, grown by vapour diffusion in hanging drops, were used as seeds (Thaller *et al.*, 1981) in subsequent crystallization experiments, set up in depression slides or in 1 mm glass capillaries. Crystals of sizes up to 0·8 mm × 0·5 mm × 0·2 mm could be

† Abbreviation used: SBA, soybean agglutinin.

0022–2836/84/120723–03 $03.00/0

obtained by this method after 14 to 20 days at room temperature (22°C). Addition of manganese or calcium ions did not affect the crystallization process. Washed and dissolved crystals retained the agglutinating activity and were indistinguishable from native SBA in electrophoresis on sodium dodecyl sulphate/polyacrylamide gel.

Preliminary precession photographs and diffractometer measurements show the crystals to be monoclinic, space group $C2$ with unit cell dimensions: $a = 118{\cdot}6$ Å, $b = 88{\cdot}9$ Å, $c = 165{\cdot}9$ Å and $\beta = 103{\cdot}0°$. The density of the crystals, as measured by density gradient in Percol solution (Pharmacia, Sweden), is $1{\cdot}133$ g/cm^3 ($1{\cdot}004$ g/cm^3 for the mother liquor), which corresponds to a molecular weight of 127,000 in the asymmetric unit (see eqn (8) of Matthews, 1974) and a V_m value of $3{\cdot}4$ Å^3/dalton (Matthews, 1968). The V_m value derived from the density measurement is in good agreement with the value of $3{\cdot}5$ Å^3/dalton obtained if one tetramer of 120,000 M_r is assumed in the asymmetric unit. Such a V_m value suggests that about 35% of the volume of the asymmetric unit is occupied by the protein, and is within the range of values compiled by Matthews (1968). The SBA crystals diffract to at least $2{\cdot}8$ Å on a rotating anode (Elliott GX-6), last for about 120 hours in the beam at room temperature and are therefore suitable for high-resolution X-ray analysis. A search for heavy-atom derivatives is in progress.

This work was supported by a grant from the Israeli Academy of Sciences. B.S. is a recipient of a Charles Clore fellowship.

Department of Structural Chemistry
The Weizmann Institute of Science
Rehovot 76100, Israel

B. SHAANAN
M. SHOHAM
A. YONATH

Department of Biophysics
The Weizmann Institute of Science
Rehovot 76100, Israel

H. LIS
N. SHARON

Received 20 November 1983

REFERENCES

De Boeck, H., Lis, H., Sharon, N. & Loontiens, F. (1983). In *Proc. 15th FEBS Meeting, Brussels, Belgium*, Abstr. S-05-TH-233, p. 213.

Dorland, L., Van Halbeck, H., Vligenthart, J. F. G., Lis, H. & Sharon, N. (1981). *J. Biol. Chem.* **256**, 7708–7711.

Gordon, J. A., Blumberg, S., Lis, H. & Sharon, N. (1972). *FEBS Letters*, **24**, 193–195.

Hemperly, J. J. & Cunningham, B. A. (1983). *TIBS*, **8**, 100–102.

Jaffe, C. L., Ehrlich-Rogozinski, S., Lis, H. & Sharon, N. (1977). *FEBS Letters*, **82**, 191–196.

Lis, H. & Sharon, N. (1973). *Annu. Rev. Biochem.* **42**, 541–574.

Lis, H. & Sharon, N. (1978). *J. Biol. Chem.* **253**, 3468–3476.

Lis, H., Sela, B. A., Sachs, L. & Sharon, N. (1970). *Biochim. Biophys. Acta*, **211**, 582–585.

Lotan, R., Siegelman, H. W., Lis, H. & Sharon, N. (1974). *J. Biol. Chem.* **249**, 1219–1224.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Matthews, B. W. (1974). *J. Mol. Biol.* **82**, 513–526.

Roth, J. (1980). *Acta Histochem.* **22**, 113–121.

Sharon, N. & Lis, H. (1982). In *The Proteins* (Neurath, H. & Hill, R. L., eds), 3rd edit., vol. 5, pp. 1–144, Academic Press, New York.

Thaller, C., Weaver, L. H., Eichele, G., Wilson, E., Karlson, R. & Jansonius, J. N. (1981). *J. Mol. Biol.* **147**, 465–469.

Edited by R. Huber