The Purification and some Properties of Wheat-Germ Agglutinin

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Wheat-germ agglutinin is a lectin with a specificity for *N*-acetylglucosamine sites on cell surfaces. It was shown by Burger & Goldberg (1967) that, in common with several other lectins, it preferentially agglutinates cells, in tissue culture, that had been transformed by chemicals or by viruses. They partially purified the protein by a procedure involving $(\text{NH}_4)_2\text{SO}_4$ precipitation and chromatography on Sephadex G-75, and claimed it to be a glycoprotein. Ozanne & Sambrook (1971) further purified wheat-germ agglutinin by chromatography on DEAE-Sephadex. However, when we followed their procedure we found that the product was separable into several bands when examined by polyacrylamide-gel electrophoresis at pH 8.5 either in the presence or in the absence of sodium dodecyl sulphate. The purification procedure that we developed involves extraction of wheat germ with water, precipitation of the protein with $(\text{NH}_4)_2\text{SO}_4$ and then successive chromatography on columns of DEAE-cellulose, SE (sulphoethyl)-Sephadex and QAE-(quaternary aminoethyl) Sephadex. On SE-Sephadex at least three active proteins were separated and were found to have very similar amino acid compositions; one of these was examined in detail.

Wheat-germ agglutinin, from its behaviour on ion-exchange columns, appears to be a basic protein with an isoelectric point between pH 9.0 and 9.5. Unlike most lectins, and contrary to previous reports, it is not a glycoprotein; glucosamine and galactosamine were not detected in acid hydrolysates, and there was no reaction in tests for neutral sugar. The amino acid composition is unusual: about one residue in five is half-cystine and almost one in four is glycine, and the number of hydrophobic residues is low. No other lectin has been reported with such a high cystine content, and the only other proteins with such a high content of cystine are the high-sulphur keratins of mammalian hair (Gillespie *et al.*, 1968). From electrophoresis in 15% polyacrylamide gel in the presence of sodium dodecyl sulphate a molecular weight of about 36000 was estimated for the native protein.

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Intermediates in the Reversible Dissociation of Apoferritin

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Horse spleen apoferritin has a molecular weight of 444000 and consists of 24 chemically identical polypeptide chains of molecular weight 18500 (reviewed by Crichton, 1972*a,b*). The maintenance of this quaternary structure is an important functional property of apoferritin.

Crichton *et al.* (1972) have shown by sedimentation-velocity techniques that apoferritin can be dissociated into subunits by extremes of pH. The dissociation occurs over the pH ranges 2.8–1.6 and 10.6–13.0, and both processes can be fitted to sigmoidal curves with mid-points, corresponding to equal concentrations of a 'fast' 17S component and a 'slow' 2–3S component, of 2.16 and 11.62 respectively. The low-pH dissociation is reversible, and the reassociation occurs at higher pH values than the dissociation (3.0–4.3) and follows a sigmoidal curve with mid-point at pH 3.76.

The reversible dissociation has now been examined by gel filtration on calibrated columns (1.5 cm × 150 cm) of Sephadex G-75, G-100 and G-150 with 10 mM-glycine-HCl and glycine-sodium acetate buffers of pH 1.5–4.5. Samples were dialysed for 24 h against column buffer before chromatography for the dissociation studies. For the reassociation, exposure to pH 1.5 for 1 h followed by dialysis for 24 h against two changes of column buffer was used. During the dissociation I observed on Sephadex G-75 columns what appear to be dimers (mol.wt. 37000) and on Sephadex G-100 and G-150 columns both dimers and tetramers (mol.wt. 70000), the latter in small amounts, in addition to the oligomer (which is excluded in the void volume) and monomer. On reassociation these same two intermediates are observed. Rechromatography of any of these components gave an elution profile similar to that obtained with apoferritin at that pH. Although the dissociation and reassociation are found to occur over the same pH range as in the previous study (Crichton *et al.*, 1972), the ratio of oligomer to monomer + dimer + tetramer observed by gel filtration is considerably lower than that found by sedimentation techniques.

It seems therefore that dimers and tetramers occur as intermediates both in the dissociation of apoferritin and in its reassociation. Whether they are also intermediates in the assembly of apoferritin from nascent polypeptide chains remains to be established.