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.

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The Catalytic Activity of Apoferritin

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Ferritin is the major iron-storage protein of mammals. The iron, amounting to some 4500 atoms in the full ferritin molecule, is present within the protein shell as a ferric hydroxyphosphate micelle, and can be removed by chemical reduction to give apoferritin, which has the same electrophoretic and immunological properties as ferritin (Mazur & Shorr, 1950) and crystallizes in a form isomorphous with ferritin (Fankuchen, 1943). It is, however, more susceptible to proteolysis (Crichton, 1969, 1971) and has a greater degree of ordered structure, as determined by optical rotatory dispersion and circular dichroism, than ferritin (Wood & Crichton, 1971; Listowsky *et al.*, 1967).

It has been suggested that apoferritin itself may act as a ferroxidase that catalyses the oxidation of Fe^{2+} to Fe^{3+} with concomitant formation of ferritin (Niederer, 1970), and some evidence in support of this view has been advanced (Niederer, 1970; Macara *et al.*, 1972). We have examined this process under a variety of conditions of pH and ionic strength and find that in 0.1M-cacodylate–borate buffers of pH 5.0–6.0 the autoxidation of Fe^{2+} with molecular O_2 as electron acceptor is considerably accelerated by the addition of apoferritin. The reaction can be followed by observing the increase in absorption at 420nm due to the formation of Fe^{3+} or by measuring the loss of Fe^{2+} by means of the $\alpha\alpha'$ -bipyridyl reaction. The product is ferritin-like material containing Fe^{3+} that migrates with the protein on polyacrylamide gels, sediments as a broad peak in the ultracentrifuge with a sedimentation coefficient considerably higher than that of apoferritin and has the electron-microscopic appearance of ferritin. 'Native' apoferritin, prepared by density-gradient centrifugation of ferritin, is as active as apoferritin prepared by chemical reduction. No other protein tested so far has any catalytic activity.

The progress curve is hyperbolic and the initial velocity increases proportionately with increasing apoferritin concentration. With respect to Fe^{2+} concentration the kinetics appear to be standard

Michaelis–Menten. The pH-dependence of the reaction and its kinetics will be described.

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A Proposed Structure for *Streptomyces griseus* Protease A

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Previous work (Johnson & Smillie, 1971, 1972) on *Streptomyces griseus* protease A (isolated from Pronase) has shown it to be an Asp-Ser-Gly serine protease that appeared to be more similar in amino acid sequence to the α -lytic protease of *Myxobacter* 495 (Olson *et al.*, 1970) than to any other serine protease. From further studies on the peptic, tryptic, chymotryptic, α -lytic, thermolytic and CNBr peptides of *Strep. griseus* protease A a complete amino acid sequence has been elucidated that accounts for all the amino acid residues of the protein.

On alignment of this sequence with those of α -lytic protease, porcine elastase and bovine α -chymotrypsin, it was found that, like α -lytic protease, *Strep. griseus* protease A possesses a large insertion (18 residues) between positions 186 and 187, with large deletions at the uranyl-loop (residues 66–81), the autolysis-loop (residues 141–154) and the methionine-loop (residues 164–178) regions.

When comparisons of homology between *Strep. griseus* protease A and the other serine proteases were made for all residues, the enzyme appeared to be most similar to α -lytic protease, with 31% identity of residues and 47% similarity of residues. In the case of comparisons between the suspected internal residues in *Strep. griseus* protease A and those in α -lytic protease, porcine elastase and bovine α -chymotrypsin, higher values were observed for residue identities (31, 19 and 21% respectively) and for residue similarities (57, 41 and 39% respectively).

On the basis of these sequence alignments, and from inspection of a model of the tertiary structure of α -chymotrypsin (Sigler *et al.*, 1968) and of the