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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