The Intracellular Distribution of Acid-Labile Selenium in Rat Liver and the Possible Biological Function of Selenium and Vitamin E

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The work of Diplock, Baum & Lucy (1968) indicated that the selenium present in rat liver is in at least three oxidation states, tentatively identified as 'selenide', 'selenite' and 'selenate'. The proportion of the total selenium found to be labile on acidification ('selenide') appeared to be dependent on the vitamin E status of the animal and was rapidly oxidized *in vitro* in the absence of anti-oxidants. The present work is a study of the intracellular distribution of 'selenide' by using zonal centrifugation. We have found that, in rats given a diet containing only sufficient selenium to protect them from liver necrosis, 'selenide' formed from intraperitoneally administered $\text{Na}_2^{75}\text{SeO}_3$ is associated with two main particulate fractions, identified by marker-enzyme and other techniques as mitochondria and smooth endoplasmic reticulum, the latter being predominant. Further appreciable amounts of 'selenide' are present in the soluble fraction and some is in a fraction believed to be rough endoplasmic reticulum. In rats given a normal diet the distribution of 'selenide' appears to be non-specific during the period of our experiments.

The specificity of the incorporation of 'selenide' into mitochondria and smooth endoplasmic reticulum in rats given a low-selenium vitamin E-deficient diet supports the possibility that 'selenide' might replace sulphide at the active centre of non-haem iron proteins in these organelles (Diplock *et al.* 1968). It has been suggested that the liquid-like properties of the α -tocopherol side chain may serve to stabilize cell membranes (Lucy, 1966). We think that, in performing this function, α -tocopherol may provide stable membranes within or upon which 'selenide'-containing proteins can function. Simultaneously the hydroxylated ring structure of the molecule may provide an appropriate redox environment for the formation and maintenance of these proteins.

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Susceptibility to Proteolysis as a Probe of the Conformation of Ferritin and Apoferritin in Solution

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Ferritin, the major iron-storage protein of mammals, consists of a central core of ferric hydroxyphosphate surrounded by a shell of protein subunits. The apoferritin, of mol.wt. 460 000, is made up of identical subunits, probably 24 in all, each of mol.wt. 18 000–19 000 (Crichton & Bryce, 1970).

It has been apparent for some time that the rate of proteolysis depends on the conformation of the protein substrate, and that if the equilibrium between different conformational states characteristic of the protein is shifted by addition of a ligand that stabilizes one or more of a small number of conformations the effect will be a decrease in the proteolysis of the protein.

It is not surprising that the binding of a 70 Å-diameter micelle of iron should lead to a decrease in digestibility of protein, and it has been shown that apoferritin is digested by trypsin to about $2\frac{1}{2}$ times the extent of ferritin, and that a number of peptides are present in apoferritin digests that are absent from ferritin digests (Crichton, 1969). The effects of chymotrypsin and subtilisin were studied, and gave results that closely paralleled those found with trypsin. However with pepsin and a cathepsin D preparation from rabbit liver (kindly given by Dr A. J. Barrett) similar rates and extent of digestion were observed at pH 3.0, and the peptide patterns were not found to differ appreciably (Crichton, 1970). This could be explained by the relatively non-specific nature of the enzymes which could lead to a failure to discriminate between the liganded and non-liganded protein. With pepsin at pH 2.5 apoferritin was digested much more rapidly than ferritin and to 2–3 times the extent. This may reflect a partial unfolding of apoferritin structure at low pH which makes the molecule more susceptible to proteolysis.

An analysis of the effect of pH on the conformation of both proteins using optical rotatory dispersion and circular dichroism showed marked changes in both proteins in the pH range 3.1–2.2 which are consistent with a loss of about 10% of their ordered structure (Wood & Crichton, 1970).

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