PN 1235

Reversible decolorisation of caeruloplasmin under acid conditions

Previous workers[1,2] have observed that caeruloplasmin, the blue copper-containing oxidase of plasma, is decolorised by acid. It has now been found that under certain conditions the decolorisation is reversible.

Two batches of human caeruloplasmin were used, one prepared by a modification of the method of CURZON AND VALLET[3], the other from the American National Red Cross. Caeruloplasmin was dialysed against 0.1 M NaCl, an equal volume of 0.5 M acetate buffer (pH 3.4–5.9) or 0.25 M phosphate buffer (pH 7.0) added and the absorbancy at 605 mμ read at 1-min intervals using a Unicam SP-500 spectrophotometer with a micro-cell attachment and 1 cm light path. Between pH 7.0 and 4.75 there was no change in absorbancy in 5 min at room temperature. At lower pH values, a decline in absorbancy was observed varying from about 10% at pH 4.5 to about 70% at pH 3.5 (pH values given refer to the buffer–caeruloplasmin mixture. Due to the slight buffering effect of the protein this is less or equal to 0.2 pH unit different from the pH of the buffer itself.) During decolorisation, the residual colour changed towards green. Upon the addition of an equal volume of 1 M sodium acetate which brought the pH above 4.75, the colour returned to the initial blue and the absorbancy increased (allowing for dilution). The results of a typical experiment are shown in Table I. Percentage reversibility decreases as time of exposure to low pH increases. This indicates that both reversible and irreversible reactions occur.

TABLE I

REVERSIBLE DECOLORISATION OF CAERULOPLASMIN AT LOW pH

The caeruloplasmin used had an initial $A_{605\ m\mu}$ of 1.22. Data in the table has been corrected for dilution. *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride was used as substrate in the oxidase-activity determinations.

$A_{605\ m\mu}$ as % of initial value		Oxidase activity after sodium acetate as % of initial value
After treatment at pH 3.5	After immediate subsequent addition of sodium acetate	
100 (0)*	100	100
51 (1)	90	88
36 (3)	76	71
32 (5)	73	65

* The figures in parentheses indicate the time of treatment in min.

Reversible decolorisation suggests a reversible change in the binding of copper to caeruloplasmin. Electron-spin-resonance (ESR) studies have shown that the cupric copper of caeruloplasmin is bound to the protein in a manner distinct from that usually occurring in model copper complexes, the ESR spectrum of caeruloplasmin[4] having a particularly low hyperfine structure constant. During irreversible decolorisation by urea[5] the typical hyperfine spectrum decreases irreversibly in parallel with the decrease of the blue colour. It has now been shown that during reversible acid decolorisation the spectrum with the typical hyperfine structure disappears reversibly. This provides further evidence of a reversible change in the characteristic binding of copper in caeruloplasmin during acid decolorisation. Whether the primary stage of

the decolorisation is denaturation due to the breaking of hydrogen bonds or a direct action of H^+ on the binding of copper to protein is now under investigation. At present, the mode of copper binding in caeruloplasmin is unknown though both imidazole[6] and carboxyl groups[6,7] have been suggested as possibilities. To study this problem it is useful to have various means of modification of the molecule in the region of copper attachment. The reversible acid decolorisation may be of some value in this respect.

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An intermolecular defect of collagen in experimental lathyrism

The experimental disease, osteolathyrism, induced in a variety of animals by agents such as β-aminopropionitrile is characterized by mesenchymal deformities[1], loss of tensile strength, and dramatic increases in collagen solubility[2]. It has been proposed that the large pool of extractable collagen is derived from old insoluble fibrils transformed to an extractable state[2]. A contrary view holds that newly synthesized molecules are prevented from polymerizing to fibrils[3]. While the molecular dimensions, conformation and fibril-forming ability of lathyritic collagen seems not to be grossly altered[2] there is evidence for a failure of intramolecular cross-linking characteristic of the maturation process[4,5].

This preliminary report documents the observation that while extracted lathyritic collagen readily forms striated fibrils *in vitro* on warming to body temperature these fibrils fail to become insoluble with increasing time of incubation in the manner characteristic of normal extracted collagen[6].

Guinea-pigs ranging in size from 250–1500 g were injected daily into the peritoneal cavity with 0.5–1 mg of β-aminopropionitrile fumarate per g of body weight. The dosage was adjusted daily so as to induce a weight loss of approx. 1–10 g/day for 14 or more days. Young actively growing guinea-pigs were used for control material. The cleaned skins were extracted with cold 0.45 M NaCl. The extracted