Possible Active Sites in Nitrite Reductase and Hydroxylamine Reductases from Vegetable Marrow (*Cucurbita pepo* L.)

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In plants, nitrite reductase behaves as a single protein producing NH_3 without involving free hydroxylamine and is dependent on one-electron donors: reduced benzyl viologen or ferredoxin (see Hewitt, Hucklesby & Betts, 1968). A closely associated hydroxylamine reductase (hydroxylamine reductase 1) utilizes additionally FMNH_2 , FADH_2 (Hewitt & Hucklesby, 1966; Hewitt *et al.* 1968) and NADPH_2 . Another hydroxylamine reductase (hydroxylamine reductase 2) functions at present only with reduced benzyl viologen or dithionite. In fungi (see Nicholas, 1959) flavoproteins containing iron plus copper in nitrite reductase and manganese in hydroxylamine reductase occur. In soya bean nitrite reductase and hydroxylamine reductase were thought to be flavoproteins activated by manganese (Nason, Abraham & Averbach, 1954). Experiments with azide, 1,1'-bipyridyl, *o*-phenanthroline, 8-hydroxyquinoline, diethyldithiocarbamate or EDTA did not indicate metals in nitrite reductase or hydroxylamine reductase from marrow (Cresswell, Hageman, Hewitt & Hucklesby, 1965), but cyanide (pH 7.5) inhibited nitrite reductase by 85% at 0.1mM and hydroxylamine reductase by 55% at 0.2mM, with reduced benzyl viologen as electron donor.

CO inhibits nitrite reductase and hydroxylamine reductase 1 (reduced benzyl viologen as electron donor) irreversibly in light. Inhibition of hydroxylamine reductase 2 by CO is reversed by light. Activity of hydroxylamine reductase 2 is related to light-absorption at 399nm. This peak shifts to 415 or 433nm on reduction by reduced benzyl viologen or dithionite respectively and returns to 399nm on autooxidation. Pyridine, dithionite and 0.1M-NaOH induce peaks at 419, 524 and 557nm. CO with dithionite produces a sharper peak at 420nm. Comparable nitrite reductase preparations do not show these peaks.

The iron chelator bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) disulphonate inhibits nitrite reductase by 50% at 0.1mM and hydroxylamine reductase 2 by 50% at 1mM, with reduced benzyl viologen as electron donor and 0.3mM substrate. Iron reverses the inhibition of nitrite reductase.

The copper chelators 2,2'-biquinolyl, 2,9-dimethyl-1,10-phenanthroline, 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline and biscyclohexanoneoxalyl dihydrazone at saturation and antimycin A and 2-*n*-

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heptyl-4-hydroxyquinoline *N*-oxide (400 $\mu\text{g}/\text{mg}$ of protein) did not inhibit nitrite reductase or either of the hydroxylamine reductases appreciably.

Phenylhydrazine and semicarbazide (10mM) did not inhibit nitrate reductase or hydroxylamine reductase 1, but semicarbazide, hydrazine and isonicotinic acid hydrazide (5–50mM) inhibited hydroxylamine reductase 2 by 20–36%. A flavin component in nitrite reductase (Huzisige, Satch, Tanaka & Hyashida, 1963) was not confirmed by precipitation with acid $(\text{NH}_4)_2\text{SO}_4$. Weak unpredictable nitrite reductase activity may occur with FMNH_2 , and hydroxylamine reductase 1 is inhibited by 25–100% by 10mM-atabrine with FMNH_2 as electron donor.

Both nitrite reductase-hydroxylamine reductase 1 and hydroxylamine reductase 2 may be iron-containing proteins with possibly haem and carbonyl groups in the latter.

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Bovine Brain Creatine Phosphotransferase: Amino Acid Sequence around the Essential Thiol Groups

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The guanidino phosphotransferases form a closely related group of enzymes of similar properties and function. When creatine phosphotransferases from muscle and brain tissues of the same species are compared significant differences in their K_m values, stability, response to alkylation, amino acid composition and peptide 'maps' are observed (Eppenberger, Dawson & Kaplan, 1967; Dawson, Eppenberger & Kaplan, 1967; Thomson, Eveleigh, Laws & Miles, 1968; Atherton & Thomson, 1969). However, there is good evidence that in the region of their essential thiol groups the amino acid sequences are similar (Thomson *et al.* 1968). This has been investigated further.

Pure bovine brain creatine phosphotransferase was