Cobamides in Extracts from *Aerobacter aerogenes*

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Cobamides in extracts from a strain of *Aerobacter aerogenes* [1] isolated from river mud have been investigated. The microorganism was grown aerobically or anaerobically in a synthetic medium with glucose or glycerol as the sole source of carbon. The cobamides were extracted from the cells as cyanoderivatives using 0.033 N HCl with added KCN [2], or as coenzymes using sodium acetate buffer, pH 6.0, in the dark [3].

The cobamides were separated by paper chromatography or by paper electrophoresis followed by bioautography [4] using a cobamide-requiring bacterium (*E. coli* 113−3 (M 200)) to locate the cobamides. The results indicated that the two cobamide coenzymes, adeninyl cobamide coenzyme and 2-methyladeninyl cobamide coenzyme are present in extracts from *A. aerogenes*. Adeninyl cobamide coenzyme has previously been isolated from other bacteria [3,5], while the isolation of the naturally occurring 2-methyl compound has not been reported. The coenzymes were detected in extracts both from aerobically and anaerobically grown microorganisms with glucose or glycerol as carbon source. Interruption of the bacterial growth at different stages along the growth curve showed no significant variation in the relative amounts of the two cobamide coenzymes. The total cobamide content in the bacterial extracts showed small variations during the first stages of growth. A slight increase was found toward the end of the logaritmic growth phase.

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The Interaction of Catecholamines with Ceruloplasmin

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It has previously been shown that catecholamines can act as substrates for ceruloplasmin [1]. The rate of oxidation of catecholamines by ceruloplasmin to the corresponding "chromes" occurs at a decreasing rate: adrenaline > isopropylnoradrenaline > noradrenaline > dopamine. The interaction of catecholamines with ceruloplasmin has been studied by spectrophotometry and electron spin resonance (ESR) spectroscopy.

In air the blue coloured ceruloplasmin is rapidly decolourized by the addition of these substrates. Simultaneously the ESR signal of the copper enzyme is decreased, indicating the reduction of Cu(II) → Cu(I) [2]. During activity a steady oxidation reduction state Cu (II) ⇌ Cu(I) in ceruloplasmin is present. The rate of decoloration of ceruloplasmin by different catecholamines has been determined spectrophotometrically by a stopped flow technique at 5°C. The rate of the decoloration of the enzyme decreases in the following order: N,N-dimethyl-*p*-phenylenediamine > noradrenaline, dopamine > adrenaline, isopropylnoradrenaline > serotonine > dopa, ascorbic acid. The experiments indicate that free radicals of catecholamines are formed due to the action of ceruloplasmin.

It has been shown that these free radicals are able to oxidize $NADH_2$ [2]. The rate of oxidation of $NADH_2$ at pH 6.0, 38°C, by catecholamines decreases in the following order: N,N-dimethyl-*p*-phenylenediamine > dopamine > noradrenaline >> isopropylnoradrenaline > adrenaline. These investigations have demonstrated that alkyl substitution at the nitrogen atom of the side chain of catecholamines decreases the rate of decoloration of ceruloplasmin. In addition the oxidation of $NADH_2$ by free radicals of catecholamines is depressed by the presence of N-alkyl groups in these substances.

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