

homozygotes and that isolated from cow β -AB through DEAE cellulose columns. These results suggest that cow β -B and buffalo β -lactoglobulin may be identical. Differences, if any, must be very small, involving uncharged groups.

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Inhibition of Plasma Monoamine Oxidase by Cysteamine

CYSTEAMINE has been shown to cause a transient inhibition of diamine oxidase¹, which was explained by its coupling with pyridoxal phosphate in a thiazolidine ring. Even when linked to pyridoxal phosphate thiols may undergo oxidation to the corresponding disulphides^{2,3} in the case of cysteamine the non-enzymatic oxidation to cystamine liberates pyridoxal phosphate, and the diamine oxidase activity is restored. Because the enzyme oxidizes cystamine⁴, we have an interesting example of an inhibitor which is changed into a substrate during the reaction.

To test the suggestion that cysteamine acts as inhibitor by binding pyridoxal phosphate, we have examined its activity towards another pyridoxal dependent enzyme, plasma monoamine oxidase. This was obtained from beef plasma according to Yamada and Yasunobu⁵. The action of cysteamine on the oxidation of spermine or spermidine was examined with enzyme preparations purified up to the steps 3 and 4 of the above authors, with similar results. The enzymatic activity was determined by following the oxygen uptake in the Warburg apparatus.

Fig. 1 shows that in the presence of cysteamine the oxidation of spermidine is inhibited until all the cysteamine has been non-enzymically oxidized to the corresponding disulphide. This is clearly indicated by the diphasic behaviour of the curves of the oxygen uptake (Fig. 1, curves 3 and 4). After the oxygen consumption has reached the theoretical value for oxidation of the thiol to the disulphide, the enzyme starts to oxidize its own substrate. The complete oxidation of cysteamine at this point has been checked by the nitroprusside test. The non-enzymatic character of the cysteamine oxidation was demonstrated by incubation with a boiled enzyme (Fig. 1, curve 7). The inhibitory effect of cysteamine has also been obtained using spermine as substrate for the enzyme. The transient inhibition of monoamine oxidase by cysteamine, which is very similar to the inhibition already demonstrated for diamine oxidase, also supports the suggestion that this particular activity of cysteamine involves pyridoxal phosphate. In this respect cysteamine

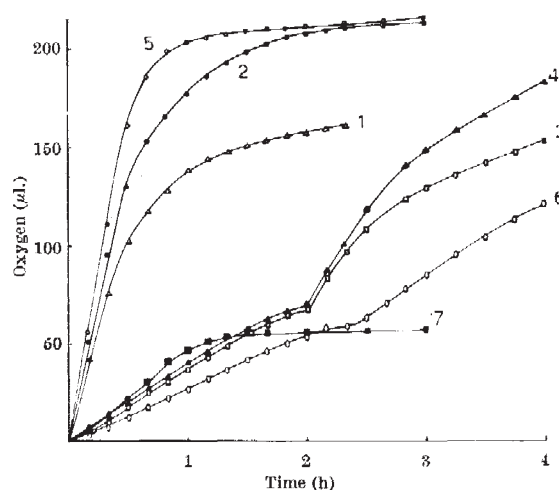


Fig. 1. 2 mg of beef plasma monoamine oxidase purified up to the step 4 of ref. 5 were incubated in 0.1 M phosphate buffer pH 7, with the following compounds: curve 1, spermidine 10 μ moles; curve 2, spermidine 20 μ moles; curve 3, spermidine 10 μ moles + cysteamine 10 μ moles; curve 4, spermidine 20 μ moles + cysteamine 10 μ moles; curve 5, spermidine 10 μ moles + cysteamine 10 μ moles; curve 6, cysteamine 10 μ moles. Temp.: 38°. Final volume, 3 ml. Gas phase: air. 2 mg of boiled enzyme were incubated in the same conditions with 10 μ moles of cysteamine (curve 7).

shows a noticeable specificity: another thiol tested, cysteine, which is unable to affect diamine oxidase⁵, is also ineffective on monoamine oxidase (Fig. 1, curve 5).

In the presence of cysteamine alone, an oxygen uptake greater than the theoretical amount for oxidation to the disulphide (Fig. 1, curve 6) indicates that cysteamine is oxidized by the enzyme. Preliminary experiments in fact have shown that the purified plasma monoamine oxidase oxidizes cysteamine, in agreement with results obtained with ox plasma by Bergeret and Blaschko⁶.

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Conversion of Cyano- and Hydroxo-cobalamin *in vivo* into Co-enzyme Form of Vitamin B₁₂ in the Rat

SINCE 5,6-dimethylbenzimidazolyl cobamide co-enzyme (DBCC) was shown to be one of the active forms of vitamin B₁₂ by Barker *et al.* in 1959 (ref. 1), its biochemical co-enzymatic activity (conversion of glutamate to methyl aspartate, methyl malonyl-CoA to succinyl-CoA, and 1,2-diols to aldehydes) and its physiological metabolism (tissue distribution, excretion and absorption) have been investigated²⁻⁴. It is now believed that vitamin B₁₂ exists as a co-enzyme form in the liver, and takes part in the trans-formation of methyl malonyl-CoA to succinyl-CoA (ref. 9). On the other hand, enzymatic synthesis of DBCC from B₁₂ derivatives has been confirmed by several workers at the bacterial enzymatic level. It has been reported that the liver and kidney homogenate of rat could convert cyanocobalamin (CN-B₁₂) to co-enzyme B₁₂ *in vitro*¹⁰. But the only report indicating that CN-B₁₂ or hydroxocobalamin (OH-B₁₂) can be converted to co-enzyme form *in vivo*, on the quantitative base, is Fenrych's short communication reporting the conversion of CN-B₁₂ into the