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A MECHANISM FOR THE ENZYMIC OXIDATION OF METHANOL INVOLVING METHOXATIN

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Summary: A novel reaction mechanism, consistent with available experimental evidence, is proposed for the oxidation of methanol and other substrates involving the new coenzyme, methoxatin, and methanol dehydrogenases from methane- and methanol-oxidizing bacteria. It also may be applicable to NADP-independent glucose oxidases.

Methoxatin is the name we have given to 4,5-dihydro-4,5-dioxo-1-H-pyrrolo [2,3-f] quinoline-2,7,9-tricarboxylic acid (I) which we believe to be the coenzyme or prosthetic group of the primary alcohol dehydrogenases found in methane- and methanol- oxidizing bacteria, a group of organisms which has attained considerable commercial importance of late for providing sources of 'single cell protein' (for example Methylophilus methylotrophus produced by ICI).

The structure of methoxatin was recently deduced from the X-ray crystal structure of its acetonide (1) and is consistent with predictions made independently by Duine, Frank and Westerling (2) who were able to infer from e.p.r. studies that the isolated coenzyme was "a quinone with two nitrogen atoms". These authors have recently provided much additional confirmatory evidence for the structure of methoxatin (3). It is clear to us that methoxatin is the coenzyme of these dehydrogenases even though this view has yet to be formally confirmed by reconstitution of the holoenzyme from apoenzyme and cofactor.

Evidence for the coenzyme nature of methoxatin is based on the observation that this compound (or its acetonide (II)) is the only low-

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molecular-weight material isolable from pure holoenzyme preparations, its release being accompanied by loss of enzyme activity. E.p.r. studies on the free and bound coenzyme also lead to this conclusion. Vigorous conditions are necessary for complete removal of the coenzyme and this suggests that it may be firmly bound to the apoenzyme, possibly covalently; in this case simply mixing the apoenzyme and cofactor would not be expected to result in a ready reconstitution of the active enzyme. We therefore propose a cyclic mechanism for primary alcohol oxidation, as follows:

Methoxatin readily undergoes nucleophilic attack at the more electrophilic carbon atom of the α-diketone system leading to addition of water or of acetone. We suggest that by a similar reaction an addition compound is formed with the amino group of a lysine residue or with ammonia or a primary amine, but to be fully consistent with the experimental evidence it is necessary to postulate that, in contrast to the reaction in solution, the steric constraints of the enzyme's catalytic site allow nucleophilic attack only at C(4). Subsequent 1,4-elimination of water from (III) (see Figure 1) will produce the quinone analogue (IV) in the active enzyme and, as it can be expected to possess similar radical forming properties to methoxatin itself, this should be capable of producing an e.p.r. spectrum. The spectra of the bound and free coenzyme will, however, differ since the former will show strong spin-spin coupling to only two protons compared to three in the latter case. De Beer *et al.*, have observed exactly this phenomenon in spectra of the dehydrogenase from *Hyphomycrobium* X (4). It is also noteworthy that the stability of the enzyme is greatly enhanced in the presence of methanol; before the cofactor can be released by 1,2-elimination it is necessary for the alcohol in the enzyme-substrate complex to exchange with water in a process which is highly unfavorable because of the larger binding constant for substrate.

With the holoenzyme assembled in the manner described, alcohol oxidation can be initiated by a 1,4-addition of the substrate giving (V).

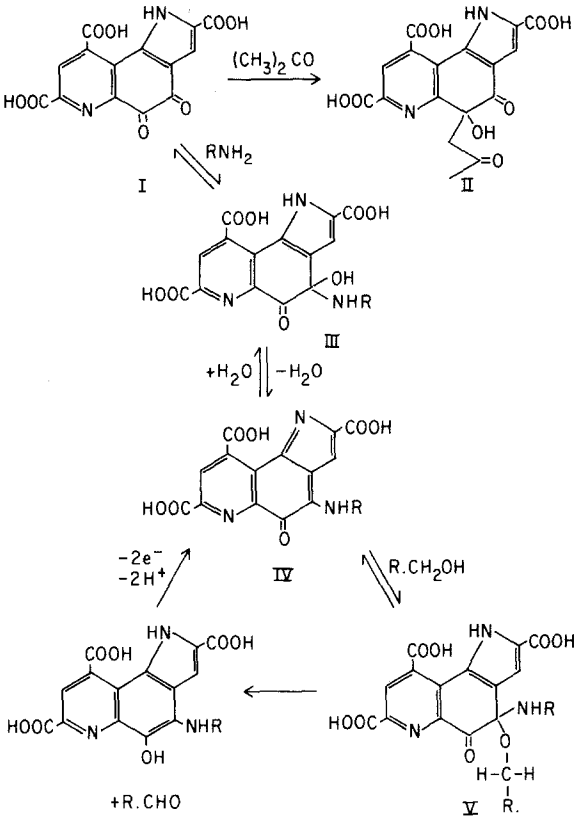

Figure 1. Scheme for the methoxatin-catalyzed oxidation of primary alcohols.

This complex can now undergo a cyclic rearrangement driven by aromatisation of the pyrroloquinoline nucleus in which an α-hydrogen atom of the alcohol is transferred to the adjacent carbonyl oxygen and the oxidized product extruded. Reoxidation of the coenzyme completes the cycle. More recently evidence has been presented (5) that methoxatin is also the coenzyme of NADP-independent glucose oxidases (6). The mechanism proposed here for primary alcohol oxidation is equally applicable to the oxidation of aldehyde hydrates to carboxylic acids and, indeed, primary alcohol dehydrogenases accept aldehydes as substrates but only those such as formaldehyde and triflouroacetaldehyde which exist predominantly as their hydrated forms in aqueous solution (7) are efficiently oxidized.

This mechanism is, we believe, both chemically feasible and consis-

tent with the experimental evidence available at present. We are currently testing its validity.

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