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INACTIVATION OF MEDIUM-CHAIN ACYL-CoA DEHYDROGENASE FROM PIG KIDNEY BY METHYLENECYCLOPROPYL-ACETYL-CoA: IDENTIFICATION OF A NEW TYPE OF FLAVIN-INHIBITOR ADDUCT

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Introduction

Medium-chain acyl-CoA dehydrogenase from pig kidney (MCADH or GAD) has previously been shown to be irreversibly inactivated by the suicide substrate methylenecyclopropylacetyl-CoA (MCPA-CoA), a metabolite derived from the poisonous amino acid hypoglycin (1). Inactivation results from covalent addition of the inhibitor molecule to the flavin coenzyme, FAD. Elucidation of the structure of the flavin-inhibitor adducts and of the detailed inactivation mechanism have been hampered by the instability of the adducts. Analysis of compounds released upon optimization of the protein denaturation procedure showed two major adducts (2,3). However, we observed that the protein denaturation procedure used did not yield the expected total amount of products (4). We thus attempted a new, drastic extraction procedure. This released a new compound, which is deduced to be very tightly, but not covalently bound to the protein. In this report we discuss some properties of this new covalent flavin-inhibitor adduct in view of its possible structure.

Results and Discussion

Medium-chain acyl-CoA dehydrogenase was completely inactivated by three equivalents of the suicide substrate MCPA-CoA per mol of flavin. Treatment of the essentially colorless protein pellet obtained upon denaturation using 90% (v/v) methanol with

4M urea and then sonication leads to the release of a bright yellow compound. HPLC analysis detects only a single chromophor with a retention time much shorter than that of any known flavin (Fig. 1). It shows the same visible absorbance spectrum as FAD, however the higher 262nm/448nm ratio indicates the presence of a chromophore which absorbs at ~260 nm, probably the CoA-residue of the MCPA-CoA moiety. The new flavin inhibitor adduct was then treated with phosphodiesterase (PDE) to remove the two AMP-moieties and subsequently with hydroxylamine (HA) to remove the CoA-residues. Fluorescence emission spectra at different pH-values showed that position N(3) of the intact isoalloxazine ring is not modified. This suggests, together with the UV/vis-properties, that the inactivator is linked to the N(10) ribityl side chain.

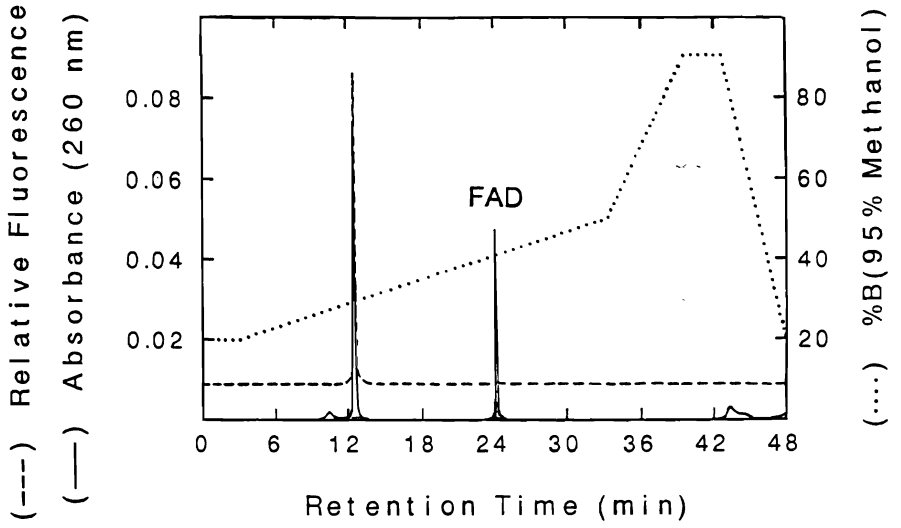


Fig. 1: HPLC analysis of the flavin-inhibitor adduct
HPLC elution profile of the chromophore containing solution obtained from the protein pellet by treatment with 4M urea/sonication. Chromatography was conducted on a RP-C18 column(Spherisorb ODS II, 5µm, 250x20mm) using the linear gradient shown at a flow rate of 4ml/min at 25°C. The elution position of FAD is shown for comparison.

The ^{1}H-NMR spectrum (Fig. 2) is also compatible with substitution at the side chain. Salient features are the presence of the 7-CH_3 and 8-CH_3 and of the 6-H and 9-H signals of the oxidized flavin.

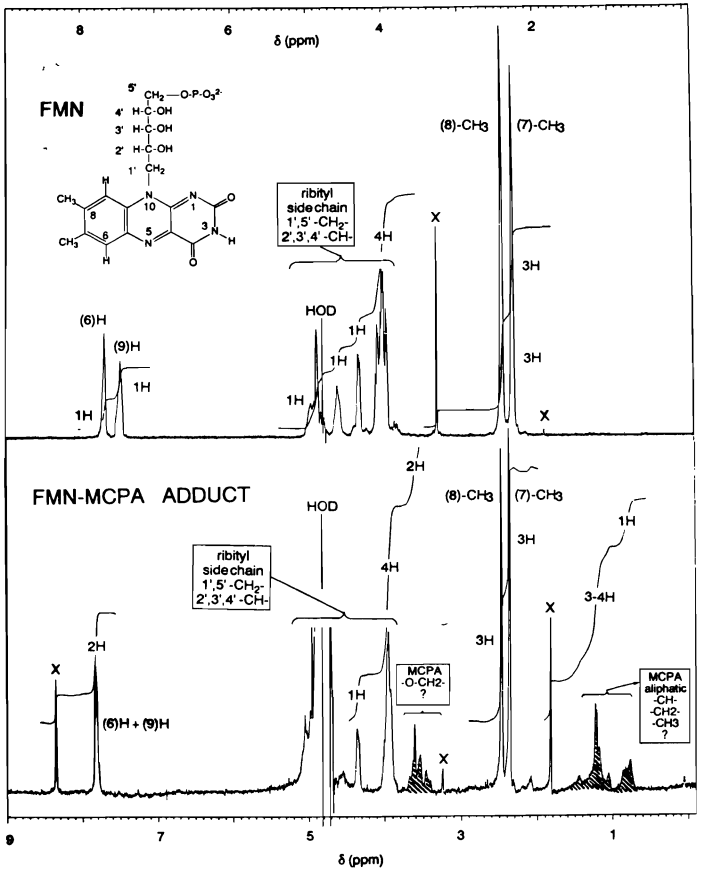
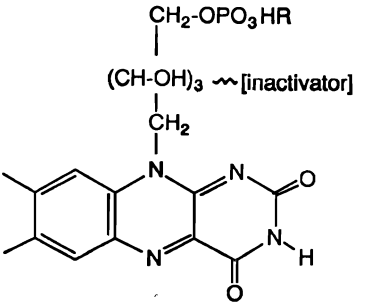


Fig. 2: ^{1}H-NMR spectrum of the PDE/HA-treated flavin-inhibitor adduct

The HPLC-purified chromophore obtained upon consecutive treatment with phosphodiesterase and hydroxylamine was dissolved in D_2O. NMR data were acquired with a 400-MHz FT-NMR-spectrophotometer (6033 pulses). Signals (X) arise from impurities. The signals assumed to arise from the MCPA moiety are shaded.

Conclusions

The novel type of adduct described here is bound extremely tightly, although not covalently to the protein, a fact demonstrated by the need of 4M urea for its release. The flavin is present in its reduced state while enzyme bound, and must be effectively shielded by access from oxygen. The sum of the evidence suggests that the inactivator skeleton is linked to the N(10) ribityl side chain, and possibly to the 2'-OH function as shown in the structure:



The information presently available does not allow the assignment of a structure to the moiety formerly belonging to the methylenecyclopropylacetate unit. It is, however, most likely that it is bridged to the flavin side chain via an ether linkage. Formation of such a bond is reminiscent of Michaelis type additions, which would have to occur at a stage in which the inhibitor would be oxidized (dehydrogenated). Formation of the adduct by secondary rearrangement of a primary adduct, can, however not be excluded at the present stage.

References

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