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INACTIVATION OF GENERAL ACYL-CoA DEHYDROGENASE FROM PIG KIDNEY BY THE SUICIDE SUBSTRATE METHYLENECYCLOPROPYLACETYL-CoA. STRUCTURE OF ONE OF THE COVALENT FLAVIN-INHIBITOR ADDUCTS.

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Introduction

General acyl-CoA dehydrogenase from pig kidney (GAD), a typical fatty acyl-CoA dehydrogenase, has previously been shown to be irreversibly inactivated by methylenecyclopropylacetyl-CoA (MCPA-CoA), a metabolite derived from the poisonous amino acid hypoglycine (1). The inactivation results from covalent addition of the inactivator molecule to the flavin coenzyme, FAD. Elucidation of the structure of the flavin inhibitor adduct(s) and of the detailed inactivation mechanism were hampered by the instability of the adducts. We have succeeded in synthesizing labelled MCPA-CoA (^{14}C at C(1) of MCPA). Inactivation with labelled inhibitor leads to incorporation of the label into the two major flavin adducts, and no reaction of the inactivator with the protein occurs. In this report we present data on the structure of the minor adduct II, which results from the attack of the inhibitor at C(6) position of the flavin.

Results and Discussion

General acyl-CoA dehydrogenase (GAD) was completely inactivated by three equivalents of the suicide substrate, MCPA-CoA, per mol of flavin. Optimization of the protein denaturation procedure (90% v/v methanol), and of the purification (HPLC) lead to the isolation of two adducts, which could be partially characterized by optical spectroscopy and ^{1}H-NMR. The major adduct, I, shows the properties of cyclic C(4a), N(5)-dihydro

flavin derivatives. This adduct is unstable and decays to oxidized flavin. Figure 1 shows the spectral properties of the second minor adduct, II, which is stable. There is a remarkable correspondence between its absorption spectrum and that of a C(6)-substituted flavin derived from an adduct formed during irreversible inactivation of D-lactate dehydrogenase from Megasphaera elsdenii by the suicide substrate D-2-hydroxy-3-butynoic acid (2,3).

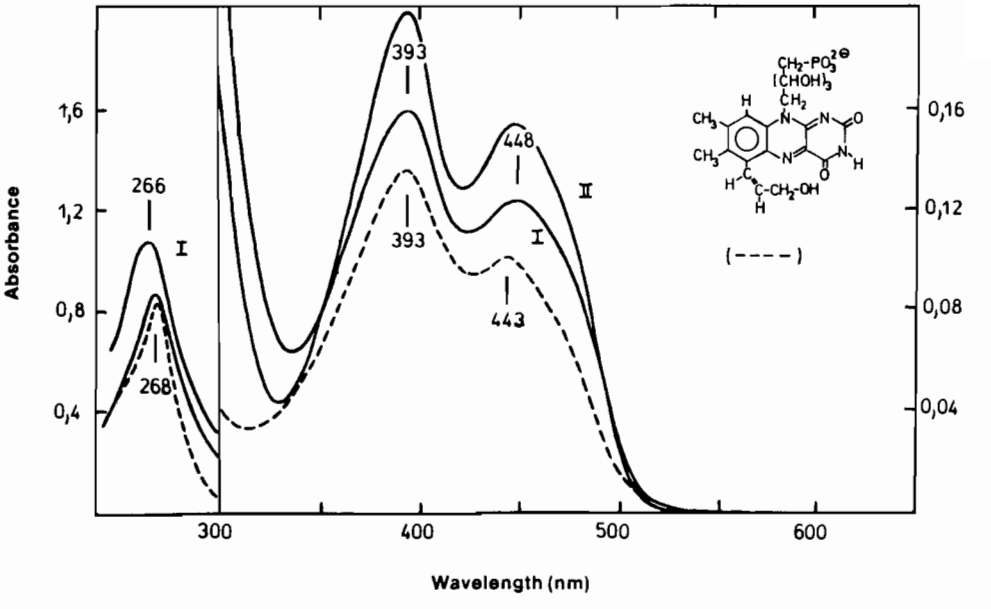


Fig. 1. Absorption spectrum of adduct II after HPLC purification
Curve I shows the spectrum of adduct II in potassium phosphate buffer pH 6.0. Curve II was obtained after treatment of adduct II with snake venom phosphodiesterase to generate the corresponding FMN analog. For comparison the spectrum of 6-(3-hydroxy-1-propenyl)-FMN is shown by curve (---).

Together with the data from the fluorescence emission spectrum of adduct II, which is compatible to that expected for a C(6)-substituted flavin, this spectral comparison suggests a covalent C(6)-flavin adduct structure. The ^{1}H-NMR-spectrum (Figure 2) is also compatible with substitution at C(6), particularly since the signal expected for the C(6) hydrogen is absent.

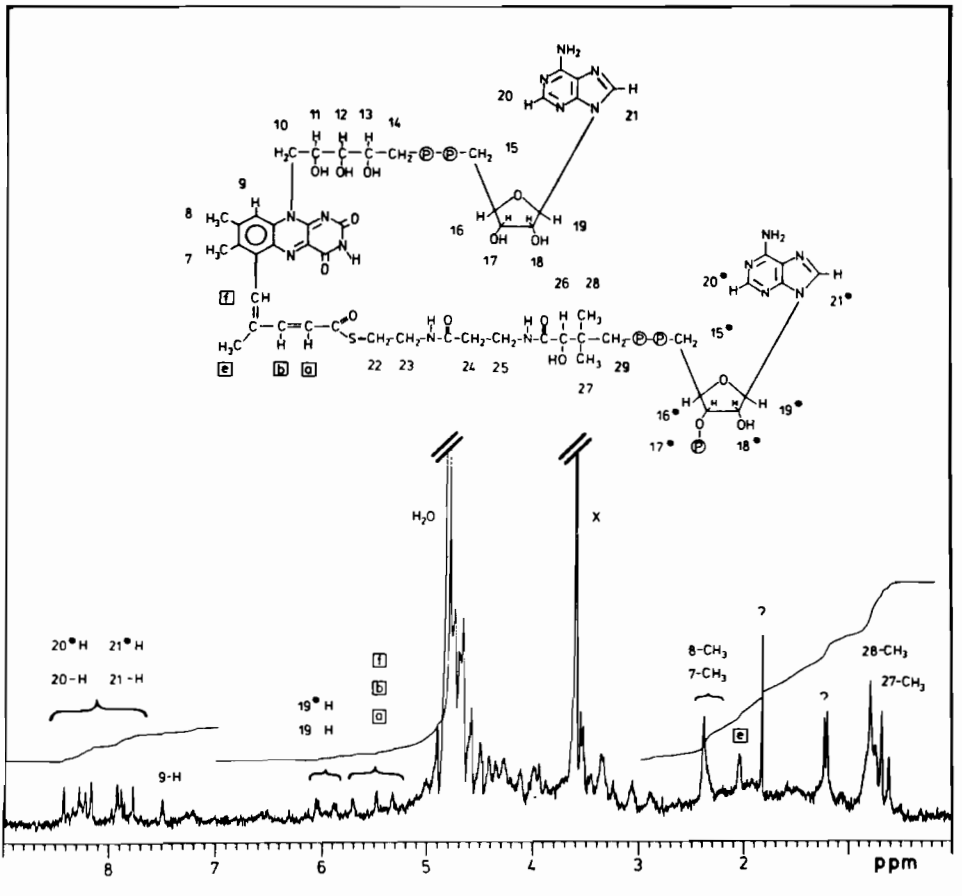
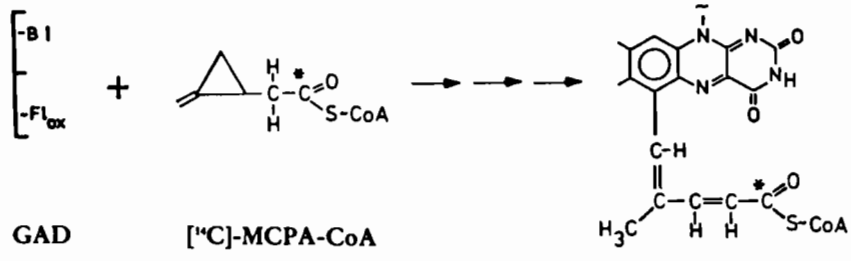


Fig. 2. ^{1}H-NMR-spectrum of adduct II and its proposed structure
The HPLC-purified chromophore was dissolved several times in D_2O and the NMR data aquired with a 250 MHz-FT-NMR spectrophotometer (1280 pulses). X and ? denote signals attributed to impurities.

Conclusions

The inactivation of GAD with radiolabelled MCPA-CoA (^{14}C at position C(1) of the MCPA-molecule) leads to the incorporation of the label into two major flavin adducts. No reaction of the inactivator with the protein occurs. The minor adduct II results from the attack of the inhibitor molecule at the C(6) position of the flavin. The structure proposed is the following:



GAD **[^{14}C]-MCPA-CoA**

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