ON THE INACTIVATION OF GENERAL ACYL-CoA-DEHYDROGENASE FROM PIG KIDNEY BY METHYLENECYCLOPROPYL-ACETYL-CoA, A METABOLITE OF HYPOGLYCIN

Studies with flavin modified enzymes

Hans-Dieter Zeller and Sandro Ghisla

Fakultät für Biologie der Universität Konstanz
D-7750 Konstanz, FRG

Introduction

General acyl-CoA dehydrogenase from pig kidney (GAD), a typical fatty acyl-CoA dehydrogenase, has previously been shown to be irreversibly inactivated by methylenecyclopropyl-acetyl-CoA (MCPA-CoA), a metabolite derived from the poisonous amino acid hypoglycin (1). Inactivation results from addition of the inactivator to the flavin coenzyme. Elucidation of the structure of the flavin adduct(s) and of the detailed mechanism of inactivation were hampered by the instability of the adduct itself. The available evidence indicates that adduct formation involves position N(5) of the isoalloxazine moiety, and probably also another flavin function such as C(6), C(4a), or C(4)=O. The goal of our work is the elucidation of the structure of the flavin-MCPA adduct. We hope that this might aid in the understanding of the inactivation mechanism of this class of suicide inhibitors.

Results and Discussion

5-Carba-5-deaza-FAD-General Acyl-CoA Dehydrogenase

Inactivation with this analog was attempted since adducts formed with it are (more) stable as compared to those formed with native enzyme (1). This modified GAD does not react with MCPA-CoA, it also does not react with

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normal substrates (2), as opposed to the very rapid reaction of 3,4-pentadienoyl-CoA, a similar suicide inactivator (3). The lack of reactivity is attributed to the very low redox potential (-310 mV) of the 5-d-FAD (4).

Iso-FAD-General Acyl-CoA Dehydrogenase

This modified enzyme shows an enzymatic activity 12% that of native GAD. It is completely and irreversibly inactivated in a way similar to that observed with native enzyme (1). This allows the exclusion of position C(6) as a point of adduct formation. The time dependence of the inactivation is very complex and does not lead to complete disappearance of the oxidized iso-FAD absorbance, suggesting inactivation due to processes other than flavin modification. However, the apoprotein prepared from inactivated iso-FAD-GAD could be reconstituted with normal FAD to yield the same complex as native enzyme, as judged from its spectral properties (absorbance ratio 260/450 nm) and its specific catalytic activity. This indicates that either protein modification does not occur, or that it is reversible.

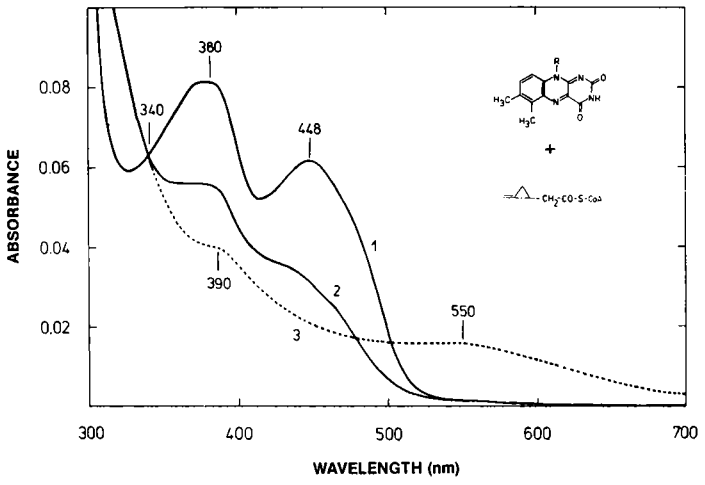


Fig. 1. Reaction of iso-FAD-General Acyl-CoA Dehydrogenase with MCPA-CoA

The enzyme, 7 μM in 0.1 M phosphate buffer pH 7.6 (curve 1) was incubated 2 h at 25° with 28.0 μM MCPA-CoA (curve 2). The reaction with 28 μM octanoyl-CoA (curve 3) is shown for comparison.

4-Thio-FAD-General Acyl-CoA Dehydrogenase

Substitution of normal FAD with 4-thio-FAD lowers the activity of GAD to 10 % the original value. 4-thio-FAD is bound only weakly by the protein. The reaction of this modified GAD with MCPA-CoA differs significantly in its course, from that observed with normal enzyme (1). First, an intermediate is formed with a half time of ~ 4 min. This species has an absorption spectrum (Fig. 2) similar to that observed upon reaction of 4-thio-FAD-GAD with octanoyl-CoA, i.e. to that of the reduced enzyme enoyl-CoA complex. In a second, slower phase, ($t_{1/2}$ ~ 110 min) a stable product is formed, the spectral properties of which are closely similar to those of an 4a-adduct formed with 4-thio-FMN lactate oxidase (5). HPLC analysis of the product(s) released upon denaturation of MCPA-CoA inactivated 4-thio-FAD-GAD shows three main peaks with retention times higher than those of 4-thio-FAD. The isolation and identification of these products is in progress.

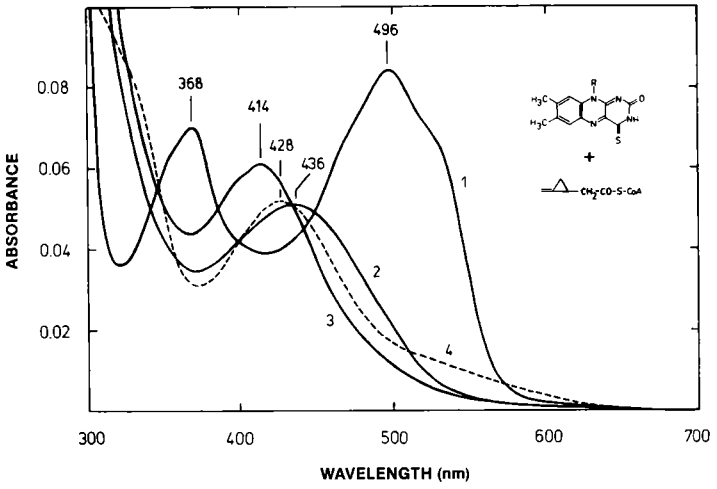
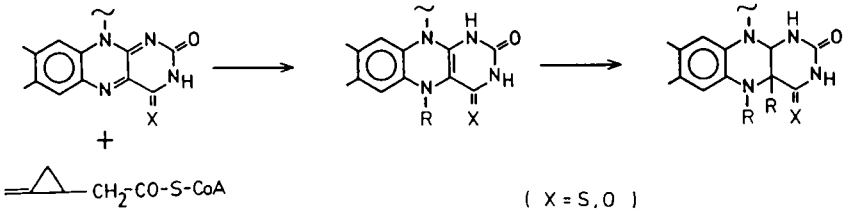


Fig. 2. Reaction of 4-thio-FAD-General Acyl-CoA Dehydrogenase with MCPA-CoA

The enzyme, 5.4 μM in 0.1 M phosphate buffer pH 7.6 (curve 1), was incubated at 25° with 22 μM MCPA-CoA. A first intermediate is formed within 30 min (curve 2). In a second, slower phase, a second species is formed, and its spectrum was recorded after 4 hr (curve 3). The product formed with 22 μM octanoyl-CoA (curve 4) is shown for comparison.

Conclusions

The kinetics of the reaction indicate that inactivation is a biphasic process. The reactivity observed with iso-FAD-GAD excludes position C(6) of the flavin as an obligatory point of adduct formation during inactivation with MCPA-CoA. When comparing with reduced 4-thioflavin models, the primary adduct most probably has an 1,5-dihydroflavin structure, the point of substitution being N(5). The primary adduct rearranges to a secondary 4a,5-dihydroflavin derivative:



The secondary adduct is unstable upon protein denaturation; it decays to products the structure of which is presently under investigation. The fact that 5-d-FAD-GAD does not react suggests that α-proton abstraction is not the only event required for initiating inactivation. This could imply that MCPA-CoA dehydrogenation is involved in inactivation.

References

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