First publ. in: Flavins and Flavoproteins / ed. D. E. Edmondson. Berlin: de Gruyter, 1987, pp. 177-180

OXIDATION-REDUCTION OF GENERAL ACYL-COA DEHYDROGENASE BY THE BUTYRYL-COA/CROTONYL-COA COUPLE. A NEW INVESTIGATION OF THE RAPID REACTION KINETICS

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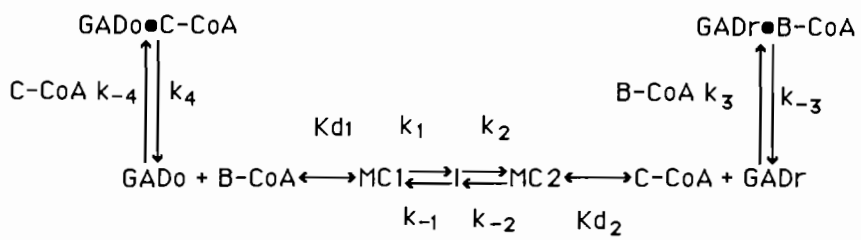
Introduction

General acyl-CoA dehydrogenase (GAD) (EC 1.3.99.3) catalyzes the insertion of a trans-double bond between C-2 and C-3 of acyl-CoA compounds. Previous mechanistic studies found that oxidized GAD reacts rapidly with butyryl-CoA to form an enzyme species which absorbs visible light (maximum at about 560 nm) and which slowly decays to a species resembling reduced enzyme (1). Rapid reaction studies (2-4) have shown that the rapid portion of this reaction is biphasic. We have undertaken a new rapid reaction investigation into the GAD/butyryl-CoA reaction mechanism. This work includes stopped-flow studies on the reactions of oxidized GAD with butyryl-CoA, reduced GAD with crotonyl-CoA, oxidized GAD with crotonyl-CoA, and reduced GAD with butyryl-CoA. We found the mechanism to be freely reversible and consistent with Scheme I.

Scheme I



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GADo is oxidized GAD, B-CoA is butyryl-CoA, C-CoA is crotonyl-CoA, GADr is reduced GAD, MC1 is a Michaelis-Menten complex between oxidized GAD and butyryl-CoA, I is a charge-transfer complex between reduced GAD and crotonyl-CoA, and MC2 is a Michaelis-Menten complex between reduced GAD and crotonyl-CoA.

Results and Discussion

Mixing butyryl-CoA with oxidized GAD (50 mM KPi pH 7.6, 4°C, anaerobically) gave a triphasic reaction, as has been seen before (1-4). The slowest phase (t1/2= 16 min) was about 250-fold slower than the preceeding phase. As such, it was considered to be a secondary process and was eliminated from consideration. The first, two, rapid phases were seen as a biphasic decrease in absorbance at 450 nm and an increase followed by a partial decrease at 560 nm, Figure 1. The faster phase resulted in formation of long wavelength absorbance. The apparent rate increased to saturation as the butyryl-CoA concentration was increased. These observations were sufficient to define steps Kd1 (37 μM), k1/k-1 (1.7 sec^{-1}/0.6 sec^{-1}) and species MC1 in Scheme I. The slower phase resulted in the partial decay of the long wavelength absorbance. The apparent rate decreased as the butyryl-CoA concentration increased. This qualitatively different type of substrate dependence indicated that butyryl-CoA entered the mechanism a second time to promote the decay of intermediate I, and led to the establishment of steps k2/k-2 (k2=0.07 sec^{-1}) and k3/k-3 (k-3=0.15 sec^{-1}).

Mixing reduced GAD with crotonyl-CoA (anaerobically) gave a biphasic increase in absorbance at 450 nm and an increase followed by a decrease at 560 nm. The fast phase resulted in formation of intermediate I. The apparent rate was directly dependent on the crotonyl-CoA concentration and led to a lower limit estimate for k-2 (220 sec^{-1}). The slower phase resulted in the formation of an oxidized enzyme spectrum, Figure 2. The apparent rate was independent of the crotonyl-CoA concentration and confirmed the value of step k-1 (0.5 sec^{-1}). The large difference in the apparent rates for formation and decay of intermediate I made determination of its spectrum possible, Figure 2. Intermediate I has very low absorbance in the 450 nm region and a pronounced long wavelength absorbance. This spectrum is consistent with the charge-transfer complex interpretation for intermediate I, where reduced GAD is the donor and crotonyl-CoA is the acceptor. Immediately after mixing, the spectrum of GAD is different from that of the starting photoreduced GAD, Figure 2.

The spectral change is consistent with the formation of a Michaelis-Menten complex and is taken as evidence for species MC2. From the overall equilibrium between species I and GADr and the values for k2/k-2, an upper limit estimate for Kd2 (250 μM) was obtained.

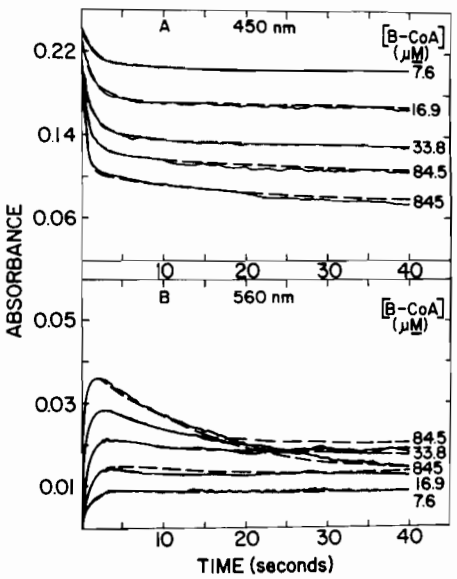

Figure 1

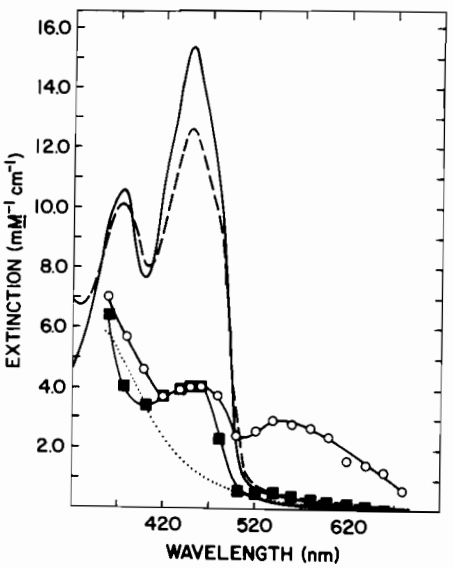

Figure 2

Figure 1 (on the left). The "rapid phases" in the reduction of oxidized GAD by butyryl-CoA. Conditions: oxidized GAD (16.4 μM) was anaerobically mixed with butyryl-CoA (15.2-1690 μM) in 50 mM KPi pH 7.6 at 4°C, using a stopped-flow spectrophotometer (2 cm optical pathlength). The indicated butyryl-CoA concentrations are after mixing. The solid lines are the measured time courses. The dashed lines are simulations based on Scheme I using the parameters described in the text.

Figure 2 (on the right). Spectra of the various species appearing during the oxidation of reduced GAD by crotonyl-CoA. Conditions: reduced GAD (16.2 μM) was anaerobically mixed with crotonyl-CoA (100 μM) in 50 mM KPi pH 7.6 at 4°C, using a stopped-flow spectrophotometer (2 cm optical pathlength). Spectra are: the initial oxidized GAD (solid line); photoreduced GAD (dotted line); the species immediately after mixing (open squares); the long wavelength intermediate (filled circles); and the final reaction mixture (dashed line).

Mixing reduced GAD with butyryl-CoA (anaerobically) gave a small, rapid rise in absorbance around the 450 nm region. The change in absorbance was similar to that seen upon the formation of MC2. This was taken to indicate formation of GADr•B-CoA, a complex originally suggested by the studies of Page and Beinert (1). The apparent rate was directly dependent on butyryl-CoA concentration and led to the description of k3 ($3x10^6$ M^{-1} sec^{-1}).

Mixing oxidized GAD with crotonyl-CoA, caused a marked red shift in the GAD spectrum, similar to that commonly encountered when ligands bind to oxidized flavoproteins. The apparent rate of formation for the complex was linearly dependent on crotonyl-CoA concentration and led to the characterization of k4/k-4 (20 sec^{-1}/$9.8x10^5$ M^{-1} sec^{-1}).

Thus we have obtained direct evidence for each enzyme species and kinetic parameter in Scheme I. The mechanism in Scheme I was then used to simulate the rate data. The measured kinetic constants presented above and a measured set of extinction coefficients served as initial estimates in making the simulations. These were adjusted by trial-and-error until a "best-fit" between the simulation and data was obtained, see Figure 1. Using this single set of kinetic and spectral parameters, all of the measured rate traces were simulated. A close correspondence between the simulated and measured data traces resulted, which makes us confident that Scheme I accurately describes GAD.

Acknowledgement

Supported by U.S. Public Health Service grant GM-11106 (V.M.), GM-26643 (C.T.) and a grant from the Deutsche Forschungsgemeinschaft (Gh 2/44 to S.G.).

References

1. Beinert, H., E. Page. 1956. J. Biol. Chem. 225, 479-497.
2. Hall, C.L., J.D. Lambeth, H. Kamin. 1979. J. Biol. Chem. 254, 2023-2031.
3. Reinsch, J., A. Katz, J. Wean, G. Aprahamian, J.T. McFarland. 1980. J. Biol. Chem. 255, 9093-9097.
4. Pohl, B., T. Raichle, S. Ghisla. 1986. Eur. J. Biochem. 160, 109-115.