SUPPLEMENTAL DATA

S1 Representative transient for the determination of the NADH dissociation rate from the R301L thioacylenzyme intermediate with MMSA as substrate. A solution of 30 µN 'activated'-MSDH, 30 µN LDH (expressed in subunit) and 20 mM pyruvate was rapidly mixed at 30 °C with an equal volume of 2 mM NAD, 0.5 mM MMSA (final concentrations). Both syringes contained 50 mM potassium phosphate buffer, pH 8.2. Under the same experimental conditions, the rate of oxidation of free NADH by LDH was shown to be 130 s^{-1}. Data collected were fit to a biphasic expression. Under these experimental conditions, no burst of NADH can be observed because the acylation step is not fast enough relative to the NADH consumption by LDH. Therefore, the first phase represents NADH consumption after its release from the thioacylenzyme–NADH complex and the second one is likely due to the reverse LDH-catalyzed reaction. The rate constants obtained for the global fitting are 1.50 ± 0.01 s^{-1} (an underestimate value for the k_{off} of NADH) and 0.010 ± 0.003 s^{-1}, respectively.

S2 Determination of the catalytic parameters of the acylation step of wild-type MSDH with PPA as substrate, under presteady-state conditions. The appearance of NADH was selectively monitored using fluorescence resonance energy transfer (FRET) on a SX18MV-R stopped-flow apparatus (Applied PhotoPhysics) at 30 °C. Following selective excitation of Trp residues at 295 nm, the fluorescence emission of NADH was measured using a 395 nm cut-off filter. In this experiment, one syringe contained 16 µN wild-type MSDH (expressed in subunit), 1 mM NAD and CoA at various concentrations and the other contained 0.5 M PPA. Data were fit to Equation 1, which yielded a $k_{obs\ max}$ of 330 ± 25 s^{-1} and a K_{app} value for CoA of 35 ± 8 µM.

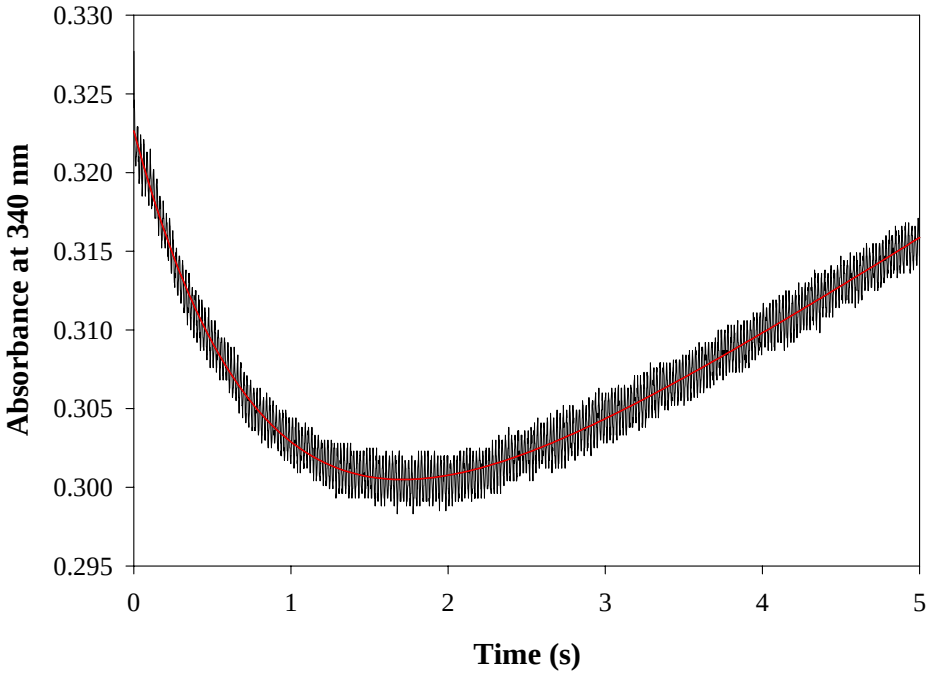
S3 Representative transient for the determination of the NADH dissociation rate from the wild-type thioacylenzyme intermediate with PPA as substrate. A solution of 30 µN 'activated'-MSDH, 30 µN LDH (expressed in subunit) and 20 mM pyruvate was rapidly mixed at 30 °C with an equal volume of 4 mM NAD, 0.25 M PPA and 100 µM desulfo-CoA (final concentrations). Both syringes contained 50 mM potassium phosphate buffer, pH 8.2. Under the same experimental conditions, the rate of oxidation of free NADH by LDH was shown to be 130 s^{-1}. Data collected were fit to a biphasic expression. The first phase represents the acylation step and the second one the NADH consumption after its release from the thioacylenzyme–NADH complex. The rate constants obtained for the global fitting are 68 ± 1 s^{-1} and 13 ± 1 s^{-1}, respectively.

S4 Determination of the catalytic parameters of the acylation step of R124L and R301L MSDHs with PPA as substrate, under presteady-state conditions. The appearance of NADH was selectively monitored using fluorescence resonance energy transfer (FRET) on a SX18MV-R stopped-flow apparatus (Applied PhotoPhysics) at 30 °C. Following selective excitation of Trp residues at 295 nm, the fluorescence emission of NADH was measured using a 395 nm cut-off filter.

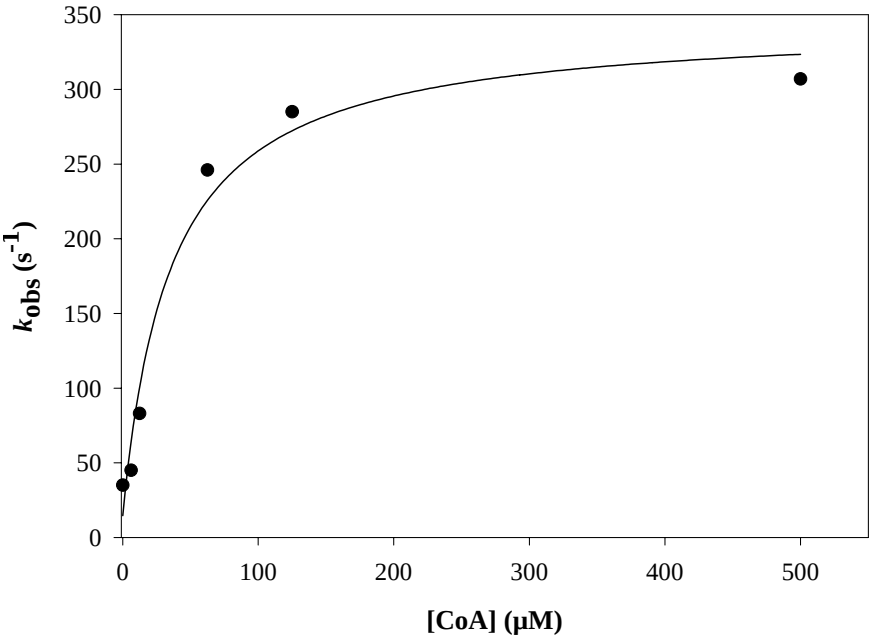
A) One syringe contained 16 µN R124L MSDH (expressed in subunit), 1 mM NAD and 250 µM CoA (final concentrations) and the other contained PPA at various concentrations. Data were fit to Equation 1, which yielded a $k_{obs\ max}$ of 110 ± 7 s^{-1} and a K_{app} value for PPA of 0.18 ± 0.03 M. The inset shows the results obtained in the presence of 16 µN R124L MSDH, 1 mM NAD, 0.525 M PPA (final concentrations) and various concentrations of CoA. Experimental data were fit against a hyberbolic expression, which yielded a K_{app} for CoA of ≈ 90 µM.

B) One syringe contained 16 μN R301L MSDH (expressed in subunit), 1 mM NAD and 250 μM CoA (final concentrations) and the other contained PPA at various concentrations. Data were fit to Equation 1, which yielded a $k_{obs\ max}$ of 117 ± 16 s^{-1} and a K_{app} value for PPA of 0.16 ± 0.05 M.

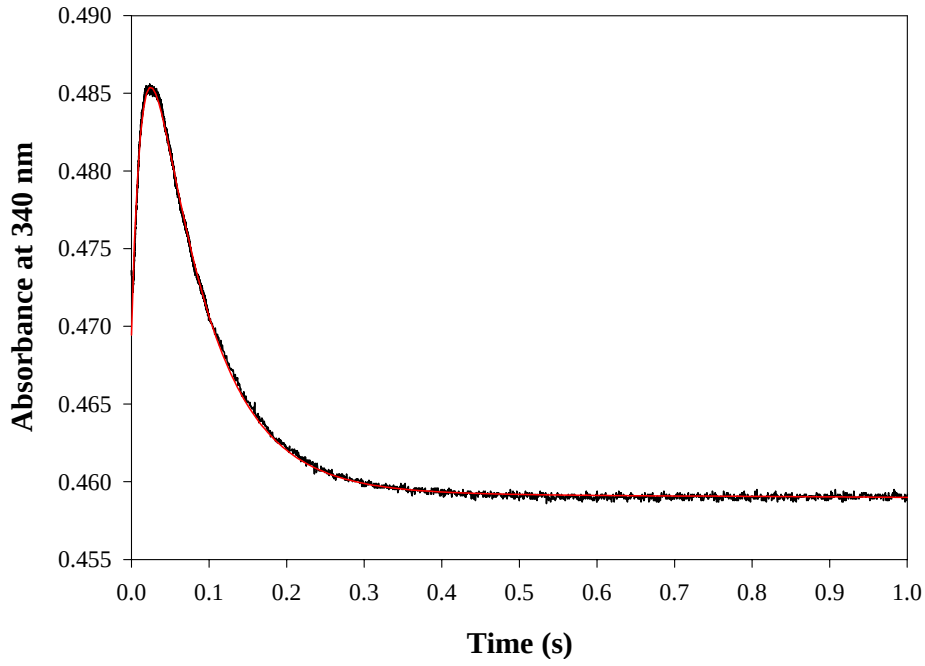
S1


Absorbance at 340 nm
0.330
0.325
0.320
0.315
0.310
0.305
0.300
0.295
0
1
2
3
4
5
Time (s)

S2


k_{obs} (s^{-1})
350
300
250
200
150
100
50
0
0
100
200
300
400
500
[CoA] (µM)

S3


0.490
0.485
0.480
0.475
0.470
0.465
0.460
0.455
Absorbance at 340 nm
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Time (s)

S4