Evidence for the Slow Reaction of Hypoxia-Inducible Factor Prolyl Hydroxylase 2 with Oxygen

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Short title: Prolyl Hydroxylase 2 Reaction with Oxygen

Figure S1. Determination of rate constants for 2OG conversion to succinate and CODD hydroxylation

As seen in Figure 2 and described in the main text, LC-MS was used to monitor the time-dependent conversion of 2OG to succinate (at 5°C) in the presence (black circles) and absence (white circles) of CODD (shown on a logarithmic scale); MALDI MS was used to quantify CODD hydroxylation with time (red circles). In order to determine rate constants, data were fitted by the equation, $f=y0+a*(1-^{(-b*x)})$, using SigmaPlot™. Apparent first-order rate constants of (0.018 ± 0.0014) s^{-1} and (0.0006 ± 0.0001) s^{-1} were obtained for succinate production in the presence and absence of CODD, respectively. An apparent first-order rate constant of (0.013 ± 0.003) s^{-1} was obtained for hydroxylation of CODD.

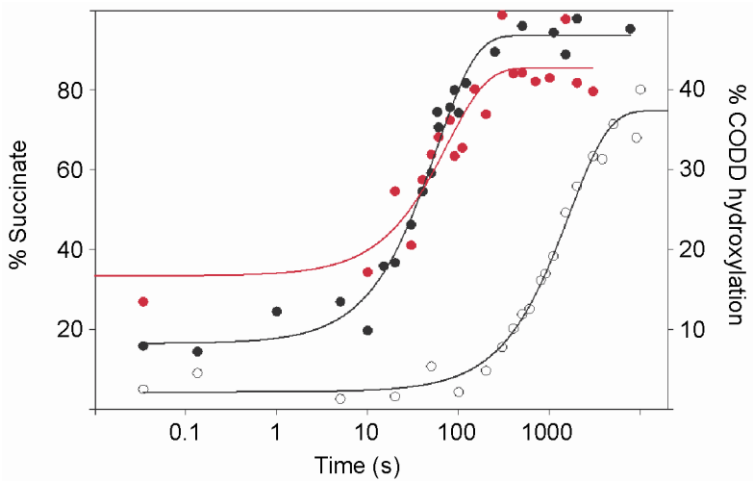
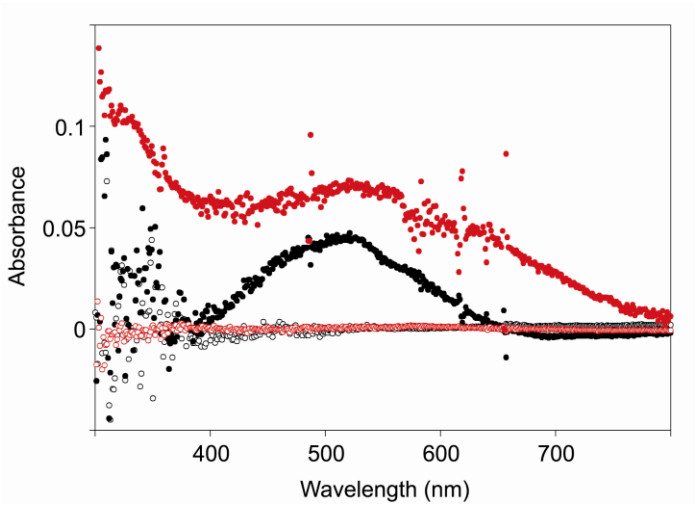


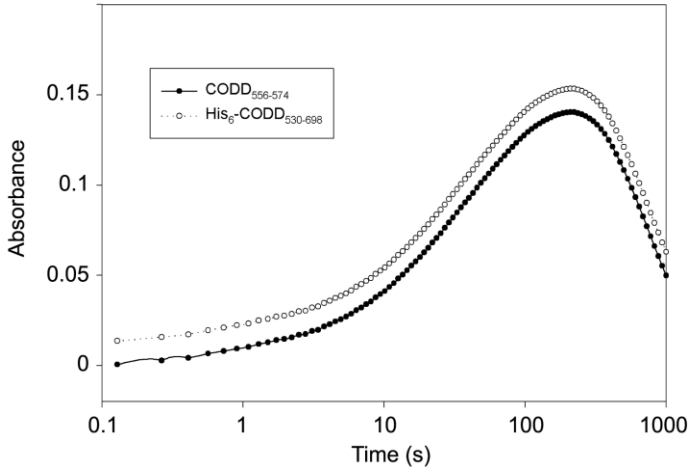
Figure S2. The PHD2.Fe.2OG and PHD2.Fe.2OG.CODD complexes demonstrate characteristic spectral features.



Addition of saturating 2OG to anoxic PHD2:Fe(II) (red open circles) and PHD2:Fe(II):CODD (black open circles) led to formation of the assigned PHD2:Fe(II):2OG (red filled circles) and PHD2:Fe(II):2OG:CODD complexes (black filled circles), with absorbance features at λ_{max} 527nm and 521nm, respectively. All spectra shown are 'blanked' against the spectrum of anoxic PHD2:Fe(II). Such features have been previously observed in

other 2OG-dependent oxygenases and shown to arise due to a metal-to-ligand charge-transfer band upon bidentate 2OG coordination to the active site Fe(II) [1]. A change in λ_{max} from ~530nm to ~520nm is often observed upon prime substrate binding.

Figure S3. UV-visible absorption spectra comparing rates of formation for the 320nm species in the presence of HIF-1$\alpha_{556-574}$ CODD peptide and His_6-$HIF_{530-698}$ CODD protein substrates.



Oxygen-saturated buffer solution was rapidly mixed with a PHD2:Fe(II):2OG:CODD complex, where CODD was either a 19mer peptide ($CODD_{556-574}$, black circles) or a longer HIF-1α protein substrate (His_6-$CODD_{530-698}$, white circles), which has a greater affinity for PHD2 [2, 3]. UV-visible absorption spectra monitored over time (shown on a logarithmic scale) demonstrated comparable rates of formation and degradation of the 320nm species ($0.06s^{-1}$ and $0.001s^{-1}$, respectively) in the presence of both substrates. This indicates that the slow rate of formation of this species observed in the presence of $CODD_{556-574}$ is not due to inefficient substrate binding.

References

1. Pavel EG, Zhou J, Busby RW, Gunsior M, Townsend CA & Solomon EI (1998) Circular dichroism and magnetic circular dichroism spectroscopic studies of the non-heme ferrous active site in clavaminate synthase and its interaction with alpha-ketoglutarate cosubstrate. *J Am Chem Soc* **120**, 743-753.
2. Ehrismann D, Flashman E, Genn DN, Mathioudakis N, Hewitson KS, Ratcliffe PJ & Schofield CJ (2007) Studies on the activity of the hypoxia-inducible-factor hydroxylases using an oxygen consumption assay. *Biochem J* **401**, 227-234.
3. Koivunen P, Hirsilä M, Kivirikko KI & Myllyharju J (2006) The length of peptide substrates has a marked effect on hydroxylation by the hypoxia-inducible factor prolyl 4-hydroxylases. *J Biol Chem* **281**, 28712-28720.