2-Oxoglutarate regulates binding of hydroxylated hypoxia-inducible factor to prolyl hydroxylase domain 2†


Prolyl hydroxylation of hypoxia inducible factor (HIF-α), as catalysed by the Fe(II)/2-oxoglutarate (2OG)-dependent prolyl hydroxylase domain (PHD) enzymes, has a hypoxia sensing role in animals. We report that binding of prolyl-hydroxylated HIF-α to PHD2 is ~50 fold hindered by prior 2OG binding; thus, when 2OG is limiting, HIF-α degradation might be inhibited by PHD binding.

The chronic response to limiting O2 in animals involves upregulation of multiple genes as enabled by the αβ-heterodimeric hypoxia inducible factors (HIFs). Under normoxia, HIF-α subunits are efficiently degraded by proteasomes; in hypoxia, HIF-α subunits accumulate, so enabling the αβ-HIF complex to promote expression of HIF target genes, including genes for biomedically important proteins, such as the vascular endothelial growth factor and erythropoietin. There is thus a considerable interest in the therapeutic manipulation of the HIF system (Fig. S1, ESI†). Evidence has been presented that the PHDs act as hypoxia sensors, including by the manifestation of an unusually slow reaction with O2, PHD catalysis has the potential to be regulated by Fe(II) and 2OG availability, as well as by succinate and other TCA cycle intermediates.

Fig. 1 Outline role of PHD-catalysed HIF-α hydroxylation.

† Electronic supplementary information (ESI) available: Details of experimental procedures and additional experiments. See DOI: 10.1039/c8cc00387d
observation of an apo-tPHD2-2OG complex, consistent with 2OG binding to the Fe(n) in catalysis. Analysis of an equimolar mixture of apo-tPHD2, Fe(n), 2OG, and CODD manifested masses corresponding to complexes of tPHD2 Fe-CODD-OH and tPHD2-Fe-CODD-OH-succinate, but not any observable quaternary complex with 2OG (Fig. 2). These results indicate that CODD and hyCODD bind to apo-tPHD2/tPHD2-Fe (Fig. 2).

Having shown that the interaction between tPHD2 and CODD is preserved in the gas phase, we then carried out solution studies. NMR studies using 15N-labelled PHD2181–402 showed that CODD and hyCODD bind with approximately equal affinity to apo-PHD2181–402 and PHD2181–402Zn (i.e. both saturated apo-PHD2181–402 and PHD2181–402Zn at 2.7-fold excess) (Fig. 3A and B). The spectrum of the PHD2181–402Zn-2OG-CODD complex was observed as reported. By contrast, hyCODD only showed weak binding to PHD2181–402Zn-2OG (Fig. 3C). The 1H–15N HSQC spectra of apo-PHD2181–402 hyCODD and apo-PHD2181–402 Zn-hyCODD are similar in the presence of excess hyCODD (Fig. 3D). The overall results imply hyCODD binding is blocked by the presence of 2OG; as anticipated, binding of CODD to PHD2-Zn is not hindered by 2OG.

To investigate the relative affinities of hyCODD and CODD to tPHD2, a fluorescence polarisation assay with N-terminal fluorescein-tagged CODD (CODD*) was developed (Fig. 3A, ESIF). Consistent with the NMR and MS data, CODD and hyCODD bind to apo- and metallated-tPHD2 equally strongly (within a 2-fold difference). With tPHD2-Zn-2OG, hyCODD was observed to bind ~50-fold less strongly than CODD (Fig. S5, ESI†). Isothermal calorimetry results showed that both CODD and hyCODD bind with similar affinity to apo-tPHD2 (9.4 ± 4.5 and 4.6 ± 1.4 μM, respectively); by contrast, CODD, but not hyCODD, was observed to bind to tPHD2-Zn-2OG (K_D = 1.8 ± 0.4 μM) (Fig. S6, ESI†). Addition of hyCODD displaced 2OG from tPHD2-Zn-2OG as observed by NMR, implying hyCODD and 2OG compete for binding to the metal complexed tPHD2 (Fig. S7, ESI†). CODD does not displace 2OG, consistent with the FP observations (Fig. S5, ESIF) and the binding requirements of the catalytic cycle (Fig S2, ESIF†).

Mutation of genes encoding for TCA cycle enzymes are observed in tumours with consequent effects on metabolite levels, resulting in proposed inhibition of human 2OG oxygenases, including the PHDs, so promoting tumorigenesis. The effects of TCA cycle intermediates (Fig. S8, ESIF†) on CODD/hyCODD binding to tPHD2-Zn were investigated by NMR; of the tested compounds, only fumarate and succinate were observed to bind to tPHD2-Zn, consistent with previous reports. By contrast with 2OG, the binding of fumarate or succinate was only weakly disrupted by hyCODD (Fig. S9, ESIF†), implying the ‘additional’ carbonyl of 2OG has a role in hindering hyCODD, but not CODD, binding to the tPHD2 metal complex. The observation of simultaneous binding of succinate and hyCODD to tPHD2 is consistent with the MS results (Fig 2d).

Analysis of crystallographic data of tPHD2 HIF-α complexes indicates the metal chelated 2OG C1 carboxylate may hinder hyCODD, but not CODD, binding (Fig. 4). By contrast, modelling of succinate binding (using tPHD2 structures and those of succinate complexed with other 2OG oxygenases) indicates that it will not manifest a steric clash with hyCODD (Fig. S10, ESIF†). Crystallographic analysis implies that in the tPHD2-Mn-2OG-ODD
The results reveal 2OG binding hinders the binding of hyCODD, but not CODD/NODD, to the tPHD2 metal-2OG complex. Thus, competition with 2OG can promote release of prolyl hydroxylated HIF-α from tPHD2 (and by implication other PHD/HIF-α isoform combinations), so enabling HIF-α degradation.4,5 Once the trans-4-hydroxyproline is formed, the ‘gauche’ stereoelectronic effect biases the conformation towards the C4-exo form, as observed in the hyCODD-pVHL complex.4,5,17 Formation of the C4-exo conformation at the tPHD active site may thus promote a clash between the hyCODD alcohol and the 2OG C1 carboxylate, which is involved in Fe(a) chelation (Fig. S10, ESIF). Hydroxylated prolyl HIF (hyHIF)-α is upregulated in many tumour cells.26 Under cellular circumstances where there is accumulation of hyHIF-α (e.g. due to mutations to the gene encoding for pVHL as occurs in the VHL disease),27,28 or if iron (e.g. in anaemia) or 2OG availability is limiting (e.g. potentially due to mutations in the genes encoding for isocitrate dehydrogenase),29,30 cellular formation of the PHD-Fe-hyHIF-α or PHD-hyHIF-α complexes may become substantial, with consequent potential limitation of the HIF-mediated hypoxic response. The results thus suggest a negative feedback mechanism for PHD activity, which is linked to TCA cycle metabolism.

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Conflicts of interest
The authors declare no conflicts of interest.

Notes and references


