Minireview

Structure and function of PCD/DCoH, an enzyme with regulatory properties

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Abstract The bifunctional protein PCD/DCoH is both an enzyme involved in the phenylalanine hydroxylation system and a transcription coactivator forming a 2:2 heterotetrameric complex with the nuclear transcription factor HNF1. The discovery of a bacterial homologue and the expression pattern during *Xenopus* embryogenesis suggest a regulatory function not only restricted to HNF1. The crystal structures of the tetrameric rat and the dimeric bacterial PCD/DCoH have led to the proposal of substrate and HNF1 binding sites. The saddle-shaped β-sheet surfaces of the DCoH dimers likely represent binding sites for as yet unknown macromolecular interaction partners. Possible mechanisms for DCoH-induced transcriptional regulation are discussed in the light of the three-dimensional structures.

Key words: Homeodomain; Tetrahydrobiopterin; Transcription activator; X-ray structure

1. Introduction

Bifunctional proteins with combined catalytic and regulatory properties are rare [1], but there are well established cases, e.g. the BirA protein of the *E. coli* biotin operon which acts as a repressor and synthesizes its own co-repressor [2,3]. In contrast to the biotin repressor/synthase case, however, the connection between the enzymatic activity of pterin-4*a*-carbinolamine dehydratase (PCD) and its regulatory function as dimerisation cofactor of HNF1 (DCoH) is not well understood. PCD is involved in the regeneration of tetrahydrobiopterin (BH4), an essential cofactor of phenylalanine hydroxylase (PAH) and other mono-oxygenases, and catalyses the conversion of 4*a*-hydroxytetrahydrobiopterin to quinoid-dihydrobiopterin [4–8]. Certain human diseases like a mild form of hyperphenylalaninemia [9] and the depigmentation disorder vitiligo [10] are linked to a lack or deficiency of PCD activity. This leads to an accumulation of 7-substituted pterins, which act as inhibitors of PAH [11] and thereby interfere with the phenylalanine catabolism and the biosynthesis of melanin pigments.

The bifunctional character of PCD became obvious when its protein sequence was shown to be identical with that of DCoH, a protein which had previously been found to stabilize the dimeric homeodomain transcription factor HNF1 and to enhance its transcriptional activity [12–14]. The discovery of a bacterial homologue of PCD/DCoH [15] and the temporal and spatial expression pattern of DCoH in *Xenopus* [16] suggest that DCoH also functions as a transcriptional regulator in an environment free of HNF1.

2. Three-dimensional structure of PCD/DCoH

The crystal structure of recombinant PCD/DCoH from rat liver was determined independently by us and Alber's group at 2.7 and 3.0 Å resolution, respectively [17,18]. The 103-residue protein folds into a compact, single domain consisting of three α-helices packed from one side against a four-stranded antiparallel β-sheet (Fig. 1A). In this open-faced α/β sandwich structure the helices run almost parallel to the β-strands, giving rise to a hydrophobic core formed by helices 2 and 3 and β-strands 2–4. Consistent with its tetrameric oligomerization state in solution [19,20] PCD/DCoH forms tetramers with 222 symmetry in the crystals (Fig. 1B). Each monomer contributes one helix (α-2) to a central, antiparallel 4-helix bundle representing the major oligomerization interface. An additional interface between monomers A/B and C/D involving the β-strands leads to the formation of 8-stranded intersubunit β-sheets in the A/B and C/D dimers. Thus, the tetramer is properly described as a dimer of dimers. The A/B (C/D) dimers are further stabilized by intensive hydrophobic contacts of the α-2 helices mediated through the stacking of Phe residues and the surface buried upon association is about one third larger than for the alternative A/C (B/D) dimers. Hence by several criteria the A/B dimers are more stable and are likely to be the interaction partners for HNF1 (see below).

The concave surface of the eight-stranded β-sheet with exposed hydrophobic residues flanked by two protruding loops on either side is reminiscent of the saddle-shaped TATA-binding protein [21–24] (Fig. 2A) and has initiated speculations about nucleic acid binding to DCoH. The topology and the surface properties of the two proteins are, however, clearly different and do not reveal closer similarity to either TBP or the RNP RNA-binding motif. Nevertheless, the shape and surface properties of the molecular saddle make it a likely interaction surface for a macromolecule.

Recently, we have solved the high-resolution X-ray structure of the *Pseudomonas aeruginosa* PCD/DCoH homologue (Ficner et al., unpublished). Interestingly, the *P. aeruginosa* protein crystallizes as a dimer and also exists as a dimer in solution (Sauer et al., unpublished results). Compared to the rat PCD/DCoH it has a 11-residue N-terminal and 3-residue C-terminal extension and displays a 33% sequence identity. On the monomer level the two structures superimpose with a root mean square deviation of 2.2 Å for 100 common C_α-atoms, excluding the N-terminal residues. A close superposi-

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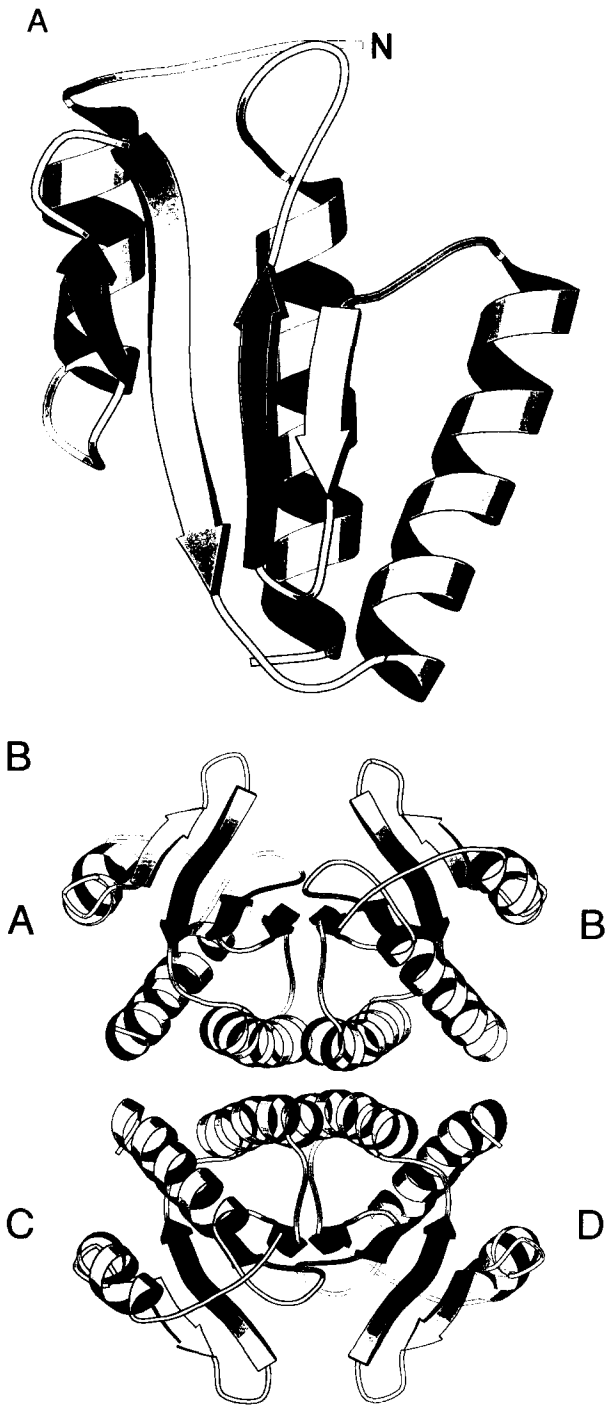

Fig. 1. Ribbon presentation of the rat PCD/DCoH monomer (A) and tetramer (B) structures. The sequential fold of the monomer is α-β-β-α-β-β-α. In the tetramer each monomer contributes its α-2 helix to the common four-helix bundle.

tion is found in the putative active site region. However, the relative orientation of the monomers in the dimer, which corresponds to the A/B dimer in the rat protein, has changed significantly resulting in a 10 Å change of the distance between the two stirrups of the molecular saddle (Fig. 2B). The absence of tetramers can be rationalized by sequence changes of residues located on the α-2 helices, which are critical for tetramer stabilization in the rat structure. These sequence changes, including N43K, T50N, R51A and K58A, are probably also responsible for the failure to detect HNF1-DCoH complex formation, when the two proteins are coexpressed in *E. coli* (see below). The N-terminal extension of the bacterial protein, which is located next to helix α-2, is partially disordered.

3. Active site of PCD/DCoH

There is as yet no direct crystallographic confirmation for the location of the active site in PCD/DCoH, e.g. in the form of an enzyme-inhibitor complex. However, strong support for the site proposed independently by two groups based on the structure of rat PCD [17,18] has been provided by the X-ray structure determination of the *P. aeruginosa* protein (Ficner et al., unpublished) and structure-based site-directed mutagenesis experiments (Köster et al., unpublished). The proposed pterin-binding site is located close to the dimer interface in a cleft between loops connecting helix α-2 with β-strand 3 and strand 4 with helix α-3 (Fig. 2A). It is lined by a number of conserved residues including H61, H62, P63, H79 (the numbering scheme refers to the rat protein [14]), some acidic residues and and a tyrosine (tryptophan in *P. aeruginosa*) contributed from the neighbouring subunit (Fig. 3). H61 and H62 have been shown to be critical for enzymatic activity and are apparently activated by ion-pair formation with carboxylates (E57, D88). The aromatic residue contributed from the neighbouring subunit also appears to be important for activity and is presumably involved in subtrate binding (Köster et al.,

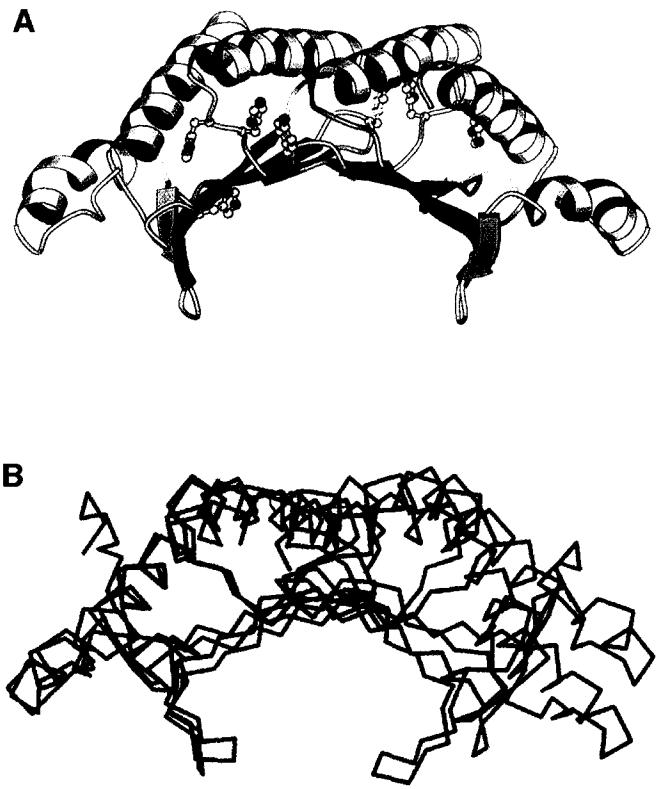

Fig. 2. (A) Ribbon presentation of the PCD/DCoH dimer. The side chains of the active site residues His-61, His-62, His-79 and Tyr-69 are shown. (B) Superposition of the Cα backbone of the PCD dimers from rat (red) and *P.aeruginosa* (blue).

Fig. 3. Alignment of the amino acid sequences of PCD/DCoH from rat/human liver, *Xenopus*, yeast and *P. aeruginosa*. The yeast protein has not yet been functionally charaterized. Conserved residues involved in the enzymatic function of PCD/DCoH are highlighted.

unpublished). The observed quenching of tryptophan fluorescence on pterin-binding to rat PCD [5] can be explained by an energy transfer process involving Trp-65. The active site of PCD/DCoH is located at the edge of the saddle-shaped surface of the dimer and one cannot rule out that pterin binding affects the association of a macromolecule to this surface. In this context, it is interesting to note that complex formation with HNF1 has no effect on the dehydratase activity of PCD/DCoH (Rhee et al., unpublished).

4. Interaction with HNF1

HNF1 (hepatocyte nuclear factor 1, also called HNF-1α or LFB1) and v-HNF1(also known as HNF-1β or LFB3) are members of a family of transcription factors which bind to DNA as homo- or heterodimers and regulate the transcription of many genes preferentially expressed in liver (for a review see [25–27]). Recently, HNF1 was shown to be required for the expression of phenylalanine hydroxylase in mice [28]. DNA-binding by HNF1 is mediated by the N-terminal 281 residues which are sequentially organized in three distinct domains: A short, 32-residue dimerization domain, a region distantly related to the POU A-box and an atypical homeodomain, whose structure is known [29]. The latter two domains are both necessary and sufficient for specific DNA-recognition, while the dimerization domain increases the DNA-binding affinity [30].

PCD/DCoH copurifies with HNF1 isolated from rat liver nuclei and, given the known dimeric nature of HNF1, Mendel et al. could show by co-immunoprecipitation experiments that the HNF1/DCoH complex formed by in vitro coexpression is a 2:2 heterotetramer [12]. This was later confirmed by gel-filtration experiments with complexes obtained by co-expression of the proteins in *E. coli* (Ficner et al., unpublished). Using various truncated versions of HNF1 it was demonstrated that the N-terminal dimerization domain is necessary for complex formation [12].

As discussed above the DCoH dimer present in the 2:2 heterotetrameric complex is most likely the saddle-shaped dimer shown in Fig. 2. The mammalian PCD/DCoH, on the other hand, exists as a very stable homotetramer in solution and thus dissociation into dimers is required for complex formation. How then does DCoH interact with HNF1 and how is the complex formed?

The X-ray structures suggest two potential interaction sites for HNF1: The antiparallel α-2 helices on one side, and the concave β-sheet surface on the opposite side of the saddle-shaped DCoH dimer. The interaction with the β-sheet appears less likely, since mixing of the proteins does not yield any complex despite the fact that the β-sheet surfaces are solvent exposed and freely accessible in the DCoH tetramer [17]. Interaction of the dimerization domain with the α-2 helices, on the other hand, could lead to the formation of an energetically favourable mixed 4-helix bundle. This notion is supported by NMR data showing that the dimerization domain of HNF1 folds into two helical segments and possibly forms a 4-helix bundle [31]. With respect to the formation of the HNF1/DCoH complex in vivo one can only speculate. As mentioned above, simple mixing of the proteins does not lead to complex formation and preliminary results do not support the possibility of a destabilization of the DCoH tetramer by a substrate-induced conformational change. There could be other, as yet unknown factors affecting the dimer-tetramer equilibrium of DCoH or, alternatively, one could conceive of a co-translational or folding-dependent event.

5. Effects of DCoH on nucleic acid binding by HNF1

As the name indicates, binding of DCoH stabilizes HNF1 dimers. The evidence for this comes from in vitro experiments showing that the exchange of HNF1 subunits is blocked in HNF1/DCoH complexes, while HNF1 homo- or heterodimers can easily exchange subunits [12]. Consistent with a stabilizing effect of DCoH is the significantly increased denaturation temperature of the DCoH/HNF1 complex compared to HNF1 alone (Rhee et al., unpublished). The stabilization of the HNF1 dimer does not seem to affect the DNA-binding affinity, but recent experiments using solid-phase DNase I footprinting suggest that DCoH enhances the stability of HNF1/DNA complexes (Rhee et al., unpublished). The observed increased resistance against salt-induced dissociation and significantly longer dissociation off-rates, however, do not appear to be the result of direct DCoH-DNA contacts as judged by the failure to detect any UV crosslinks. The

DNase I-footprinting technique also suggests that DCoH apparently promotes interactions of HNF1 with weak, variant target sites. A very surprising result was the detection of RNA-binding by HNF1, which is apparently suppressed by DCoH (Rhee et al., unpublished).

6. Other interaction partners of DCoH

As outlined above, structural and biochemical evidence argues against binding of HNF1 in the molecular saddle surface of the DCoH dimer. On the other hand, its particular shape and surface properties strongly suggest that it represents an interaction interface for a macromolecule. A logical consequence is that there must be other macromolecular interaction partners, either proteins or nucleic acids. This view is strongly supported by the identification of the bacterial homologue of PCD/DCoH in *P. aeruginosa* as a product of the *phhB* gene, which apparently is required for the expression of phenylalanine hydroxylase gene (*phhA*) from the same operon [15]. While these results suggest a regulatory role for the bacterial DCoH, they leave open the question of whether this is achieved through direct interaction with the nucleic acid or through another protein. Reports of preliminary results suggesting that mammalian DCoH can restore steady-state levels of *phhA* mRNA in bacterial cells carrying *phhB* truncation mutations, and that PhhB, the bacterial PCD/DCoH, facilitates expression of HNF1-dependent genes in mammalian cells, have triggered speculation on an underlying common mechanism for regulation [18]. Cross-species complementation of transcriptional control proteins is, however, very unusual and needs further verification.

Additonal evidence for other interaction partners of PCD/DCoH comes from a recent analysis of the spatial and temporal expression pattern of *Xenopus* DCoh (XDCoH) during embryogenesis [16]. XDCoH is as a maternal factor present in the egg before HNF1 is expressed and located in the nuclei as early as zygotic transcription starts. The tissue distribution of XDCoH during embryogenesis and the location in the nuclei of cells devoid of HNF1 suggest that it has a role in differentiation and acts as a transcriptional regulator in cell types not containing HNF1 [16].

7. How does DCoH regulate transcription?

Knowledge of the 3D structure of DCoH has provided important insights into substrate binding and the interaction with HNF1, but it is clear that further structural and biochemical data are needed to unravel the mechanism of transcriptional regulation by DCoH. The unexpected structural features of DCoH have, however, initiated new thoughts and hypotheses on how this may work. Considering the presently available structural and biochemical data several scenarios are possible. The enhancement of HNF1-dependent gene expression could be induced by: (1) Modulation of HNF1 binding to DNA. The enhanced stability of HNF1/DCoH-DNA complexes and the recognition of variant HNF1 target sites argue in favour of such a mechanism (Rhee et al., unpublished). DCoH may, for example, promote cooperative binding to distant, weak HNF1 binding sites by mutual contacts in the saddle surfaces of the DNA-bound HNF1/DCoH tetramers, as it is seen in the crystal structure [17]. (2) Modulation of HNF1-RNA interactions. New experimental data suggest that HNF1 binds RNA and that this binding is blocked by DCoH (Rhee et al., unpublished). (3) Binding of another protein in the saddle of the DCoH/HNF1 heterotetramer.

DCoH appears to have a regulatory function not only in the context of HNF1-dependent transciption, but also in bacteria and in *Xenopus* cells not expressing HNF1 [16,17]. Given the (remote) structural similarity of DCoH with RNA-binding motifs, direct interaction of DCoH with RNA would therefore be an attractive model providing a universal mechanism for the regulatory function. So far, however, there is no experimental support for such a model, but further investigations and a more thorough search for potential binding sites are necessary.

Finally, it should be pointed out that there is still no established link between the enzymatic activity of PCD/DCoH and its regulatory function. A plausible link would be the effect on the expression of phenylalanine hydroxylase, which is regulated by PCD/DCoH in procaryotes and by HNF1 in mammals [15,28]. However, experimental evidence for a pterin-dependent transcriptional regulation by DCoH is lacking, but it would not be surprising if such a coupling does indeed exist.

8. Conclusions and perspectives

The crystal structures of mammalian and bacterial PCD/DCoH combined with structure-based site-directed mutagenesis and new biochemical data have shed light on the enzymatic mechanism and the interaction with HNF1. However, there are central issues which remain unresolved: How is the DCoH/HNF1 heterotetramer formed in vivo, given the high stability of the DCoH tetramer? What is the mechanism of stimulation of HNF1-dependent transcription? What are the interaction partners of DCoH in cells and organisms not containing HNF1? Is there a direct interaction of DCoH with DNA or RNA? Finally, one would like to know if and how the regulatory function of PCD/DCoH is coupled to its enzymatic activity.

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