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Crystal Structure of an Asymmetric Complex of Pyruvate Dehydrogenase Kinase 3 with Lipoyl Domain 2 and its Biological Implications

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Summary

A homodimer of pyruvate dehydrogenase kinase (PDHK) is an integral part of pyruvate dehydrogenase complex (PDC) to which it is anchored primarily through the inner lipoyl-bearing domains (L2) of transacetylase component. The catalytic cycle of PDHK and its translocation over the PDC surface is thought to be mediated by the “symmetric” and “asymmetric” modes, in which the PDHK dimer binds to two and one L2-domain(s), respectively. Whereas the structure of the symmetric PDHK/L2 complex was reported, the structural organization and functional role of the asymmetric complex remain obscure. Here, we report the crystal structure of the asymmetric PDHK3/L2 complex that reveals several functionally important features absent in the previous structures. First, the PDHK3 subunits have distinct conformations: one subunit exhibits “open” and the other “closed” configuration of the putative substrate binding cleft. Second, access to the closed cleft is additionally restricted by local unwinding of the adjacent α -helix. Modeling indicates that the target peptide might gain access to the PDHK active center through the open but not through the closed cleft. Third, the ATP-binding loop in one PDHK3 subunit adopts an open conformation, implying that the nucleotide loading into the active site is mediated by the inactive “pre-insertion” binding mode. Altogether our data suggest that the asymmetric complex represents a physiological state in which binding of a single L2-domain activates one of the PDHK protomers while inactivating another. Thus, the L2-domains likely act not only as the structural anchors but also modulate the catalytic cycle of PDHK.

Keywords

pyruvate dehydrogenase kinase; lipoyl-bearing domain; asymmetric complex; crystal structure; pre-insertion configuration

In eukaryotes, the flow of carbon from the glycolytic pathway into the Citric Acid Cycle is controlled by the mitochondrial pyruvate dehydrogenase complex (PDC), a large macromolecular machine similar in size to prokaryotic ribosome. It consists of three major components: pyruvate dehydrogenase (E1), dihydrolipoamide transacetylase (E2) and dihydrolipoamide dehydrogenase (E3)¹. The PDC activity is regulated by phosphorylation of several E1 serine residues (Ser 264, Ser 271, and Ser 203 located on the α -chain of E1) catalyzed

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by a dedicated pyruvate dehydrogenase kinase (PDHK), an integral component of PDC. In mammals, PDHK is represented by four tissue-specific isoforms (PDHK1-4) which differ markedly with respect to their activities and regulation². On average, each complex contains 2–3 PDHK molecules bound to the inner lipoyl-bearing domains (L2) of the 60-meric E2 component^{1,3}. The association with L2 facilitates PDHK access to the multiple copies of E1 component, and also provides the means for regulation of PDHK activity³.

Consistent with the structural data presented in this paper, previous structural studies carried out by several laboratories established that PDHK forms a homo-dimer that is a likely functional state of the enzyme^{4–6} (Figure 1(a)). The PDHK protomer consists of two domains: the N-terminal, lipoyl- and pyruvate-binding regulatory domain (R-domain), and the C-terminal, nucleotide-binding domain (K-domain) also playing a key role in dimerization^{4–6}. The core of R-domain is built of four α -helices arranged as a four-helical bundle. The K-domain has an α/β sandwich fold containing the five stranded mixed β -sheet flanked on one side by four α -helices, while the opposite side comprises the dimer interface (Figure 1(a)). The loop between the two C-terminal helices of the K-domain (ATP lid, residues 301–327) is thought to control the access of ATP to the nucleotide-binding site⁷. Whereas the ATP lid was disordered in all ATP-free structures^{5,6}, it becomes partially folded and constitutes a portion of the nucleotide-binding site in the ATP/ADP-bound structures^{4–6}. In particular, the glycine-rich C-terminus of the lid, so-called G2-box⁷ forms a P-loop-like structure⁸ that provides the major interactions with the ATP phosphates^{4–6}. The C-terminal portions of PDHKs (residues 367–399⁴; and residues 385–399⁶) were missing in all L2-free structures^{4,6}. Upon binding to the L2-domains they adopt the extended conformations to form the so-called “C-terminal tails” (cross-arms) that are swapped between the PDHK subunits thereby allowing the tail of one subunit to interact specifically with the L2-domain bound to another^{5,6}.

In order to phosphorylate multiple E1 components, tightly bound PDHK must be able to move around the surface formed by the E2 core. The movement of PDHK was rationalized in terms of the hand-over-hand transfer mechanism, in which PDHK is transferred from one L2 domain to another without a complete dissociation. The PDHK/L2 complex is thus oscillating between the “symmetric” and “asymmetric” states in which the PDHK dimer binds to either two or one L2 domains, respectively⁹. The recently determined structure of the symmetric PDHK3/L2 complex (SYMC)⁵ provided deep insights into mechanism of PDHK action. However, the structural organization of the asymmetric complex, as well as its role in regulation of PDHK activities remain obscure.

To shed additional light on the structural and functional modes of PDHKs during their association with the E2 core of PDC, we have crystallized the asymmetric PDHK3/L2 complex (ASC). The resulting structure provided a snapshot of the PDHK3/L2 in the asymmetric state and revealed a number of local, but likely functionally significant, alterations of the PDHK3 structure. Altogether, our data suggest that the asymmetric binding of the L2 domain activates one of the PDHK3 subunits in the dimer, while inactivating another. The (asymmetric complex) ASC structure provides an experimental support for the previous hypothesis that the L2 domains serve not only to anchor kinase to PDC but are actively involved in switching conformations and in regulating activities of PDHKs³.

The structure of the ASC

The asymmetric unit of the ASC crystals contains two nearly identical dimers of PDHK3 bound to one L2 domain each (Table 1 and Figure 1(a)). A single L2 domain in the ASC possessing a β -barrel architecture interacts with both protomers of the PDHK3 dimer in a similar fashion to that of the SYMC⁵. It forms the major interface with one of the PDHK3 subunits (the L2-bound subunit) which, in particular, involves docking of the lipoyl-lysine moiety of L2 into

the deep hydrophobic cavity of the R-domain (Supplementary Data, Figure S1); this observation agrees well with earlier studies⁵. The L2 interface with the other L2-free subunit of PDHK3 is limited to the interactions of L2 with the extended C-terminal tails. At a first glance both the structures of the PDHK3 monomers and their dimerization mode closely resemble those of the other reported PDHK structures with a highest similarity to PDB entry 1Y80 (RMSD of 1.0 Å and 1.2 Å for the monomer and dimer, respectively). However, detailed comparative analysis of the ASC structure revealed several alterations of functionally significant structural elements as compared to both the SYMC and L2-free PDHK structures.

The PDHK subunits in the ASC adopt distinct conformations

The binding of the L2 domain induces complete folding of the C-terminal tails of the L2-free subunit that interacts with the L2 domain through its C-terminal portion. These interactions bridge the R-domains of the two PDHK subunits in the complex and result in re-orientation of the R-domain of the L2-free subunit as compared to the L2-free structures where the C-terminal tails appear to be disordered. Out of four long helices forming a core of the R-domain the position of the $\alpha 6/\alpha 7$ helix (residues 101–136; the amino acid numbering corresponds to the expressed, rather than processed, sequence) is affected by this rearrangement to the greatest extent (Figure 1(b)). Importantly, this helix forms a wall of the putative E1-substrate binding cleft (the substrate cleft, SC) located in between the R- and K-domains of the enzyme^{6,7}. The SC was also shown to accommodate the binding site for pyruvate that inhibits the PDHK activity presumably by precluding the E1-substrate binding⁶. The movement of the $\alpha 6/\alpha 7$ helix induced by the L2 binding results in an overall widening of the SC by ~ 4.5 Å that might facilitate the access of the substrate to its binding site on the L2-free subunit (Figure 2(a) and (b)). It is worth noting that nearly identical “opening” of the SC was also observed in the SYMC⁵.

However, whereas the SCs of both PDHK protomers in the SYMC exhibit open, presumably active conformations, the SC of the L2-bound subunit in the ASC retains closed configuration reminiscent of that observed in the L2-free PDHK structures^{4,6}. This is a likely consequence of the absence of the second L2 domain and the resulting flexibility of the L2-bound C-terminal tail. This tail, although partially folded in the middle providing some connecting interactions with the L2-free subunit, is disordered at the N- and C-termini thereby likely may not be able to support the tightening required to stabilize necessary rearrangements of the L2-bound subunit (Figure 1(a)).

These structural considerations suggest that the binding/dissociation of the L2 domains establishes/disrupts functional communications (through the swapped C-terminal tail) between the PDHK subunits that trigger opening/closing of the SC “gate” (the $\alpha 6/\alpha 7$ helix of the R-domain) thereby likely regulating delivery of the substrate to the active site of kinase. This hypothetical mechanism is consistent with the recent results showing that the progressive truncation of the C-terminal tails dramatically affects the PDHK activity¹⁰.

The “gate” helix of the L2-bound subunit is unwound in the middle

In all previously reported PDHK structures and in the structure of the L2-free subunit of the ASC the $\alpha 6/\alpha 7$, gate helix adopts an identical prominently bent ($\sim 30^\circ$) conformation (Figure 1(a) and (b)) that closely resembles the “uniform” state of the catalytic bridge helix of multi-subunit RNA polymerases (RMSD = 1.7 Å over 36 C $^\alpha$ atoms) (Figure 2(c))¹¹. In PDHKs, the bending is facilitated by one residue (Asp121 in PDHK3) that does not maintain an α -helical conformation thereby providing distortion and slight kink in the middle of the helix. Surprisingly, however, the center of the gate helix of the L2-bound domain in the ASC undergoes substantially more dramatic distortions, resulting in complete unwinding of one turn of the helix (Figure 1(a)). The unwound residues form a loop that protrudes out of the helical axis across the SC thereby additionally narrowing (by ~ 6 Å) the closed SC of the L2-bound

subunit (Fig. 2A and 2B). This apparently unstable conformation is maintained through hydrogen bonding between the two basic residues (Arg158 and Arg162) and unwound Asp121 (Figure 2(e)). The side chain orientations of these basic residues are strikingly distinct from those in other PDHK structures. In the L2-subunit, the Arg162 side chain occupies the space inside the protein released by the unwound residues from the gate helix, thus providing steric stabilization to the loop. On the other hand, Arg162 forms a (unique) bridging hydrogen bond with Tyr334 located on the opposite side of the SC in close vicinity to the active site of the enzyme thereby additionally stabilizing the closed conformation of the SC. Since the gate helix is not involved in formation of the crystal lattice, its unusual configuration is likely attributed to the asymmetric binding of the L2 domain that may affect conformation of the helix through a long-distance allosteric signaling mechanism which was previously proposed to regulate the activity of the enzyme^{6,12,13}. Interesting, a very similar distortion of the bridge helix was observed in bacterial RNA polymerase (Figure 2(d)), where this distortion is thought to mediate substrate loading into the active site during the transcription cycle¹¹. We speculate that transitions between the uniform and locally unwound conformations of the PDHK gate helix may play analogous role in regulating delivery of the E1-substrate to the catalytic center of kinase.

Modeling of the PDHK/E1-substrate complex

To provide further support to the hypothesis that the SC constitutes the major substrate-binding site, whose opening/closing modulates the substrate access to the active site of the enzyme, we decided to construct a structural model of PDHK in complex with the E1-target. Our analysis shows that the dimensions of the open SC in the L2-free subunit (17×8 Å using the distances between the C^α atoms) allow it to accommodate a 12-residue long α -helix in such a way that its side chains would make no steric clashes with the PDHK residues in the SC. The interior of the SC is lined by a large number of the conserved hydrophobic residues, suggesting that van der Waals interactions would likely determine the binding specificity of the substrate peptide, which therefore should possess an exposed complementary hydrophobic surface. In the E1 structure (PDB ID 1NI4), the peptide (residues 261–278) containing the two phosphorylation sites (Ser264 and Ser271) forms a loop on the protein surface that is missing both the secondary structure and specific interactions with the rest of the molecule. It therefore may readily adopt an α -helical conformation upon binding to PDHK through the induced-fit mechanism. Our modeling showed that this E1 loop folds into an amphipathic α -helix, in which the two substrate serines are located on one side of the helix, while the other side appears to be rich of the hydrophobic side chains. The following docking of the E1 structure (in which the substrate loop was modeled in an α -helical conformation) into the open SC of the L2-free subunit of the ASC (Figure 3(a)), in combination with homology modeling of ATP in the nucleotide-binding site of the K-domain (PDB entry 1Y8P), revealed that Ser264, the major phosphorylation site of E1 can be easily positioned near the PDHK active center (~3.3 Å away of the ATP γ -phosphate) without any alterations of the E1 or PDHK structures. In the final model, the hydrophobic residues of the substrate helix and SC complement each other to form the inter-molecular hydrophobic core (Figure 3(b) and (c)). Importantly, the closed SC of the L2-bound subunit appeared unable to accommodate the modeled α -helical peptide.

Though we can not rule out the possibility that the E1 substrate peptide retains the loop conformation in the E1/PDHK complex, the following structural considerations support the hypothesis of its isomerization into an α -helical configuration upon binding to PDHK. First, according to the modeling, the loop $\rightarrow$ α -helix transition does not require alterations of the E1 structure, whereas the docking of the modeled substrate α -helix into the PDHK SC produces no steric hindrance between the E1 and PDHK structures (Figure 3(a)). Second, in all biological systems specific binding of the cognate substrates requires their tight packing within the enzyme active sites to achieve substrate recognition and efficient catalysis. However, judging

by the dimensions of the SC, the E1 substrate peptide in the loop conformation would be loosely bound to PDHK, suggesting that either (i) substrate refolding into the α -helix with a perfect match to the unaltered SC (Figure 3(b)), or (ii) the SC closure, likely accompanied by significant rearrangements of the whole PDHK subunit that in turn may also affect the dimer configuration, would be required to achieve such a tight fit between the E1 and PDHK. Of these two possibilities, the E1 substrate loop refolding seems to be a more elegant and energetically favorable solution. Third, the E1 substrate peptide contains a number of the hydrophilic residues that in the loop configuration would be projected into the hydrophobic SC, thereby disturbing complementarity of the E1/PDHK interface. Finally, additional modeling demonstrated that the second phosphorylation site (Ser271) may be docked into the active position upon simple translation of the substrate helix along the SC during which the hydrophobic interface between the substrate peptide and PDHK would be maintained. It is not immediately clear how the Ser264/Ser271 exchange in the PDHK active site may be achieved without a loss in binding specificity if the substrate peptide adopts the loop conformation. Though our hypothetical model is certainly very rough and thus could not be used for the analysis of the E1/PDHK interactions at the atomic level, this model is readily testable by the biochemical techniques (i.e. site-directed mutagenesis, cross-linking, etc.) and may also facilitate design of the E1/PDHK complexes for future structural studies.

The ASC structure reveals an open conformation of the ATP lid in the L2-free subunit

Whereas the ATP lid loop is completely disordered in the L2-bound subunit of the ASC (consistent with the other nucleotide-free structures), it appears partially folded in the structure of the L2-free subunit. The ordered portion of the lid includes, in particular, the G2-box (residues 323–328) that adopts a P-loop conformation similar to that of the ATP(ADP)-bound structures⁸ where it closes the nucleotide-binding site and specifically binds the phosphates of the nucleotide through its main chain imino groups. The orientation of the G2-box in the ASC appears, however, strikingly distinct and may be designated as an “open” conformation as compared to the “closed” form observed in the ATP-bound complexes (Figure 4(a) and (b)).

For a number of other NTP-processing enzymes (for example, RNA polymerases and some aminoacyl-tRNA synthetases), loading of the NTP substrate into the active center has been shown to occur via a two-step mechanism, in which the substrate initially binds to the enzyme in an inactive, open conformation (“pre-insertion” site) followed by the isomerization of the complex into the active, closed (“insertion”) state^{14,15}. Delivery of the NTP substrate from the pre-insertion to insertion site is thought to occur upon a simple rotation of the nucleotide around a hinge located near its γ -phosphate moiety. The finding that the G2-box in the ASC maintains the active conformation capable of binding the phosphates of the nucleotide suggests that in PDHKs ATP may also bind to its binding site in the open, pre-insertion configuration preceding its delivery to the closed, catalytically competent insertion site. To test this prediction we have modeled the substrate ATP bound to the L2-free subunit of the ASC based on a simple superposition of the closed and open G2-boxes from the ATP-bound and our structures, respectively. The model reveals no steric hindrance and shows that ATP-PDHK binding in the putative pre-insertion site would be nearly as stable as that in the insertion site, relying not only on the interactions with the nucleotide phosphates but also on specific recognition of its base moiety (Figure 4(a)). This site, however, would be apparently inactive judging by large distances between the ATP α - and β -phosphates ($\sim 5.5\text{\AA}$ and $5.3\text{\AA}$, respectively) and catalytic Mg^{2+} ion, which in the catalytically active, “insertion” ATP configuration should be bound to all the phosphates of ATP and the active site residue Glu247 (Figure 4(b)). As was proposed for the other systems, in PDK3, the ATP molecule bound to the open, pre-insertion site might be loaded into the catalytic, insertion site through $\sim 90^\circ$ rotation around its γ -phosphate (Figure 4(c)). We therefore propose that, similarly to other NTP-binding enzymes, the pre-insertion

ATP-binding step in PDHKs may be utilized to achieve selection of the proper substrate prior to catalysis^{14,15}.

In the ATP-bound PDHK structures, the side chain of Tyr326 from the closed G2-box lies at the bottom of the SC, contributing to formation of the hydrophobic surface (Figure 3(b)). In contrast, in the open configuration observed in the ASC, this side chain is substantially raised, thereby blocking rather than complementing the putative E1 binding site and limiting access of the phosphorylation substrates to the active center. The open state of the ATP lid might therefore serve to obstruct the binding of the target E1 peptide in the absence of ATP suggesting that either ATP binding is prerequisite binding of the protein substrate, or that these two events occur in concerted manner.

Mechanism of the PDHK action

In summary, our results and analysis imply that in the ASC, the structure of the PDHK dimer undergoes several functionally important alterations (likely induced by the asymmetric binding of the L2 domain) that inactivate the L2-bound protomer, while activating the L2-free subunit. These alterations are unlikely to be induced by crystal packing on one hand and were not observed in the other PDHK structures on the other, suggesting that the ASC is one of the physiological intermediates that PDHK forms during its catalytic cycle and/or migration over the PDC surface.

What is the exact role of the ASC in regulation of the PDHK activities? Structural considerations suggest the two major hypotheses. First, the ASC represents one of the two functional states that mediate phosphorylation of the multiple E1 substrates. In this scenario, the SYMC would play the role of the catalytic state assuming that the two E1-substrates are processed simultaneously by the two active PDHK subunits, which are both attached to PDC through the (two) L2 domains. The ASC would be then a “transition” state that is formed during translocation of PDHK to the new phosphorylation sites. This mechanism, however, would require two steps of translocation before the next active SYMC could be formed.

Second, the ASC is the major functional PDHK intermediate on PDC (Figure 5). In this “asymmetric” mode, the catalysis would be carried out by only one L2-free PDHK subunit likely lacking tight binding to PDC (through the L2 domain), while the other, inactive L2-bound subunit would play a role of structural anchor. The two mechanisms of the PDHK traveling from one E1 substrate to another fit to the asymmetric catalytic mode. In the classic, hand-over-hand mechanism the act of catalysis might result in the structural alterations of the complex that would increase affinity of the L2-free PDHK subunit to the adjacent L2 domain, while facilitating dissociation of the L2-bound subunit from L2. This will convert catalytic subunit into the structural one and vice versa, and would allow selection of a new phosphorylation target. In the alternative, “circular” mechanism the ASC may rotate around its binding site on PDC thereby providing access of the catalytic L2-free subunit to the E1 substrates located within a radius determined by a size of the PDHK dimer. The ASC would then phosphorylate several distinct sites without swapping the functional roles of its PDHK subunits. Which of these (or some other, yet unknown) catalytic and translocation mechanisms are correct remains to be elucidated by future biochemical and structural studies.

Protein Data Bank accession code

The coordinates and structure factors for the crystal structure of the ASC have been deposited in the Protein Data Bank with accession code XXX.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

PDC	pyruvate dehydrogenase complex
E1	pyruvate dehydrogenase
E2	dihydrolipoamide transacetylase
E3	dihydrolipoamide dehydrogenase
PDHK	pyruvate dehydrogenase kinase
L2	the inner lipoyl-bearing domain of E2
R-domain	regulatory domain of PDHK
K-domain	nucleotide-binding domain of PDHK
SYMC	symmetric complex of PDHK with L2
ASC	asymmetric complex of PDHK with L2
SC	substrate binding cleft

Figure 1.

Three-dimensional structure of asymmetrical PDHK3-L2 complex. (a) The stereo ribbon diagram of the PDHK3 dimer viewed approximately down the two-fold axis. The PDHK3 subunits, domains, and key structural elements are color-coded and indicated by arrows. L2F and L2B are the L2-free and L2-bound PDHK subunits, respectively. (b) The structure of the PDHK L2-free subunit is colored from blue (<1Å) to red (>7Å) according to the deviation in the positions of the C_α atoms of the corresponding L2-free and L2-bound residues. The structural elements missing in the L2-bound subunit are indicated by the black color.

Figure 2.

The gate helices in the L2-free and L2-bound subunits. (a, b) The L2-free open (a) and L2-bound (b) configurations of the SC (marked by yellow hydrophobic residues) correspond to the uniform (magenta, (a)) and distorted (orange, (b)) conformations of the gate helix. The remainder of the subunits is shown in gray. The PDHK active site is marked by the modeled (PDB ID 1Y8P) ATP molecule. (c, d) Superposition of the L2-free (magenta, (c)) and L2-bound (orange, (d)) PDHK gate helices with the RNA polymerase bridge helix (white) observed in the uniform (c) and locally distorted (d) conformations. (e) Stereo view of the L2-bound subunit showing stabilization of the partially unwound gate helix. The hydrogen bonds are shown by magenta dashed lines. The active site is marked by ATP as in panels (a) and (b). The view in the panels (a), (b), and (e) is the same as in the Figure 1(b).

Modeling of the E1/PDHK complex. (a) The overall stereo view of the E1 α -subunit with the modeled α -helical substrate peptide (yellow) docked into the SC of the L2-free PDHK subunit. The E1 β -chain and PDHK L2-bound subunit are located far from the modeled interface and are omitted from the model for clarity. (b) The close-up stereo view of the interface between the E1 substrate α -helix (yellow) and the SC. Hydrophobic side chains of the substrate are shown in yellow, the phosphorylation site (Ser264) that is modeled in close proximity ($\sim 3.3\text{\AA}$) to the ATP (green) γ phosphate is colored in orange. The PDHK backbone and hydrophobic side chains are shown in the domain-dependent colors corresponding to those in the panel (a). The view is roughly the same as in the Figure 1(b). (c) Schematic drawing of the putative van

der Waals interactions (dashed lines) that may be formed between the E1 substrate α -helix and the PDHK hydrophobic residues in the SC. The color scheme is the same as in panels (a) and (b).

The pre-insertion model of ATP loading. (a, b) The stereo views of the inactive pre-insertion (a) and catalytically-competent, insertion (b) ATP-binding sites modeled on the L2-free subunit structure. The pre-insertion (yellow, (a)) and insertion (blue, (b)) G2-boxes may bind the pre-insertion (orange, (a)) and insertion (green, (b)) ATP phosphates in the essentially similar manner. In the pre-insertion site, the side chain of Lys134 and the main chain carbonyl oxygen of Tyr303 may form the base-specific hydrogen bonds (blue dashed lines) with ATP, while binding of the ATP base moiety may be additionally stabilized through stacking interactions with the Ile131 and Tyr303 side chains. The PDHK active site is marked by the catalytic Mg^{2+} ion (cMg, magenta sphere) and the active site residue, Glu247. The backbone of the

PDHK is shown in gray. The view is same as in Figure 1(b). (c) The insertion configuration is achieved through $\sim 90^\circ$ rotation of the pre-insertion ATP around the γ -phosphate.

Figure 5.
Hypothetical mechanism for two potential asymmetric modes of PDHK translocation.

Table 1

Data collection and refinement statistics

Data collection			
Space group	P4 ₁ 2 ₁ 2		
Unit cell dimensions (Å)	a=b=96.155 c=222.984		
Wavelength (Å)	1.0		
Resolution (Å)	30 – 2.50 (2.59–2.50) ^a		
Unique	34366 (2373)		
Redundancy	4.3 (1.9)		
I/σ ^b	12.1 (1.5)		
Rmerge (%) ^c	9.7 (42.2)		
Complete (%)	92.8 (65.4)		
Refinement		Model quality	
Space group	P4 ₁		
Resolution	30 – 2.5 (2.61–2.50)	RMSD ^f bond length (Å)	0.012
Reflections used	64645	RMSD bond angles	1.742
Rfactor (%) ^d	19.0 (30.7)		
Rfree (%) ^e	24.4 (32.3)		
Number of protein atoms ^g	12893	Ramachandran plot regions	Fraction of residues (%)
Number of Ligand atoms	22	Most favorable	85.6
		Allowed	14.1
		Generously allowed	0.3
		Disallowed	0.0

Notes to Table 1: The PDHK3/L2 complex was prepared as described previously¹⁶. Crystals were obtained using a hanging drop vapor diffusion technique by mixing 2 μl of protein solution containing the PDHK3/L2 complex (3–5 mg/ml), 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 5 mM dithiothreitol, and 2.5% (v/v) ethylene glycol with an equal volume of reservoir solution containing 100 mM histidine (pH 6.4), 30 mM EDTA, and 7% (v/v) 2,4-methyl pentane diol (MPD). Crystals were flash-frozen in the reservoir buffer containing high concentration (30%) of MPD. The crystals belong to the P4₁(3)2₁2 space group with unit cell dimensions a=b=96.15 Å c=222.98 Å. Diffraction data were collected at IMCA beamline 17 ID, APS at 100K and were processed using the HKL2000 program¹⁷. The structure was solved by molecular replacement using the AMoRe program¹⁸ and the coordinates of the SYMC (PDB entry 1Y8N), and revealed the PDHK3 dimer bound to one L2 domain in the asymmetric unit of the P4₁2₁2 space group. Further inspection of the diffraction data¹⁹ indicated that the crystals belong to the P4₁ space group and possess perfect merohedral twinning (operator h,-k,-l) coupled with non-crystallographic symmetry closely resembling the P4₁2₁2 space group. Therefore the diffraction data were expanded and the two complexes were generated to allow us the refinement in the P4₁ space group using the twinning option implemented in the CNS program²⁰. The refinement converged to a final Rfactor(Rfree) of 19.0% (24.4%) at 2.5 Å resolution.

^aThe data for the highest resolution data shell are shown in parentheses.

^bI/σ – ratio of mean intensity to a mean standard deviation of intensity.

^c $R = \sum hkl \sum j |I_j(hkl) - \langle I(hkl) \rangle| / \sum hkl \sum j \langle I_j(hkl) \rangle$, where $I_j(hkl)$ and $\langle I(hkl) \rangle$ are the intensity of measurement j and the mean intensity for the reflection with indices hkl , respectively.

^d $R_{\text{factor}} = \sum hkl ||F_{\text{calc}}(hkl)| - |F_{\text{obs}}(hkl)|| / \sum hkl |F_{\text{obs}}|$.

^eR_{free} is as for Rfactor but calculated for a test set, comprising 5% of the total number of reflections.

^fRMSD – root mean square deviation.

^gResidues missing from the model (L2-free subunit chain ID - A; L2-bound subunit chain ID - B; L2-domain chain ID - C): A1–A12, A304–A317, A401–A406, B1–B13, B137–143, B263–266, B305–B326, B383–B385, B391–B406, C127–C134, C214–C224. The protein with His-tag was used in crystallization¹⁶ that was not visible in the ED map.