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The Pyruvate Dehydrogenase Complexes: Structure-based Function and Regulation*

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The pyruvate dehydrogenase complexes (PDCs) from all known living organisms comprise three principal catalytic components for their mission: E1 and E2 generate acetyl-coenzyme A, whereas the FAD/NAD^+-dependent E3 performs redox recycling. Here we compare bacterial (*Escherichia coli*) and human PDCs, as they represent the two major classes of the superfamily of 2-oxo acid dehydrogenase complexes with different assembly of, and interactions among components. The human PDC is subject to inactivation at E1 by serine phosphorylation by four kinases, an inactivation reversed by the action of two phosphatases. Progress in our understanding of these complexes important in metabolism is reviewed.

The pyruvate dehydrogenase complex (PDC)[3] catalyzes the oxidative decarboxylation of pyruvate with the formation of acetyl-CoA, CO_2 and NADH (H^+) (1–3). The PDC occupies a key position in the oxidation of glucose by linking the glycolytic pathway to the oxidative pathway of the tricarboxylic acid cycle. In mammals, PDC plays the role of a gatekeeper in the metabolism of pyruvate to maintain glucose homeostasis during the fed and fasting states. The flux through PDC is tightly regulated in tissues under different metabolic conditions. This is accomplished by covalent modification of the rate-limiting component of the PDC involving dedicated kinases and phosphatases (4–7). PDC is also implicated to play a role in degenerative neurological diseases, obesity, type 2 diabetes, and other diseases (8–11). More recently, PDC has emerged as an enzyme of interest in cancer biology because of a switch from oxidative metabolism to aerobic glycolysis in some cancers (12–14). The focus of this review is to present recent developments on structural aspects, as well as on structure-based catalytic mechanisms of the PDCs, illustrated with *Escherichia coli* PDC (PDCec) as the simplest form and human (mammalian) PDC (PDCh) as a highly evolved form of the complex for its regulation.

PDC Components and Organization of the Complexes

The PDCs in prokaryotes and eukaryotes are composed of multiple copies of three catalytic enzymes: pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2), and dihydrolipoamide dehydrogenase (E3) (Fig. 1, *top left*). These three catalytic components work sequentially, catalyzing the oxidative decarboxylation of pyruvate with the formation of acetyl-CoA, CO_2 and NADH (H^+). The E1 is a thiamin diphosphate (ThDP)-dependent enzyme and catalyzes two consecutive steps (refer to Fig. 1, *bottom*): (i) the decarboxylation of pyruvate to CO_2 with the formation of C2α-hydroxyethylidene-ThDP (enamine) intermediate (k_2, k_3) and (ii) the reductive acetylation of the lipoyl groups covalently attached to the E2 (k_4, k_5). The E2 catalyzes the transfer of an acetyl moiety to CoA to form acetyl-CoA (k_{-7}/k_7). The transfer of electrons from the dihydrolipoyl moieties of E2 to FAD and then to NAD^+ is carried out by E3. Higher eukaryotic PDCs have an additional structural component, dihydrolipoamide dehydrogenase-binding protein (E3BP), and two regulatory enzymes, pyruvate dehydrogenase kinase (PDK, four human isoforms) (15, 16) and pyruvate dehydrogenase phosphatase (PDP, two human isoforms) (6, 17, 18) totaling 11 proteins in PDCh with all isoforms included. Additionally, there are two isoforms of the α subunit of E1h that are encoded by separate genes in most mammals (19, 20). The X-linked gene (*PDHA1* in human) encodes E1α subunit (PDHA1) present in all somatic tissues, whereas an autosomal, intronless gene (*PDHA2* in human) is expressed only in the testis. E1h in this review refers to PDHA1 protein expressed in somatic cells.

The E2 in eukaryotes and prokaryotes has a multidomain structure, comprising from the N-terminal end 1–3 lipoyl domains (L1 and L2 in E2h; L1, L2, and L3 in E2ec; ~80 amino acids each), the peripheral subunit-binding domain (PSBD, ~45 amino acids) to which E1ec and E3ec bind in PDCec or the E1-binding domain to which E1h binds in PDCh, and a large C-terminal catalytic domain (~250 amino acids) that forms the dodecahedral or cubic inner core and where acetyl-CoA is synthesized (Fig. 1, *top right*). Domains are connected by Ala- and Pro-rich hinge regions that are 20–30 residues in length. E3BP has a domain structure similar to that of E2h and is composed of

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[3] The abbreviations used are: PDC, pyruvate dehydrogenase complex; E1, pyruvate dehydrogenase; E2, dihydrolipoamide acetyltransferase; E3, dihydrolipoamide dehydrogenase; E3BP, E3-binding protein; E3BD, E3-binding domain; ThDP, thiamin diphosphate; ec, *E. coli*; h, human; bs, *B. stearothermophilus*; LD, lipoyl domain; 3-lip E2ec, wild type E2ec with three lipoyl domains; 1-lip E2ec, E2ec with one hybrid LD (LD_h); PSBD, the peripheral subunit-binding domain; PDK, pyruvate dehydrogenase kinase; PDP, pyruvate dehydrogenase phosphatase; HEThDP, C2α-hydroxyethyl-ThDP; LThDP, C2α-lactylThDP; IP, iminopyrimidine; c, catalytic.

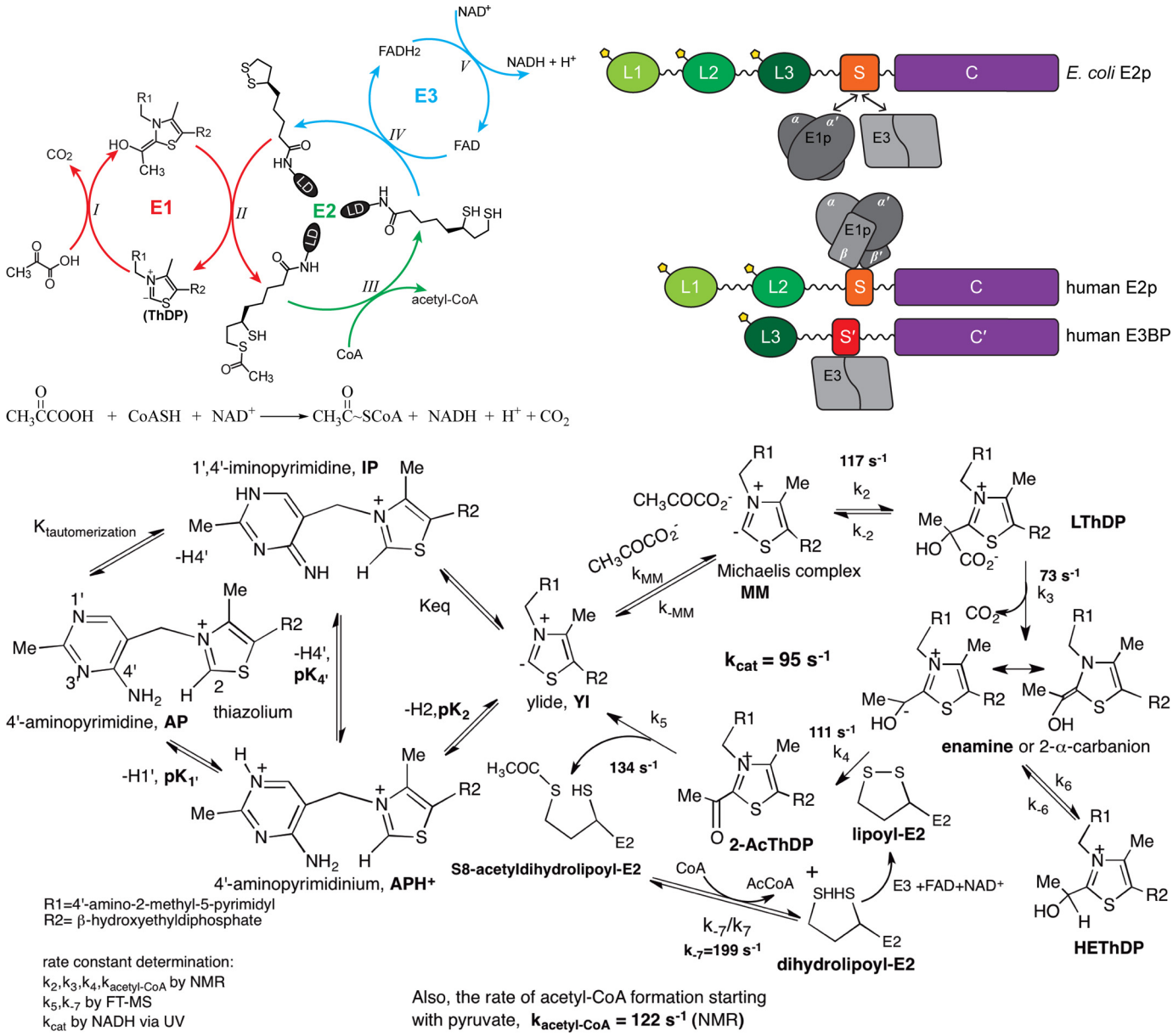

FIGURE 1. **Overall PDC reactions, E2 and E3BP domain structures, and stepwise E1 reactions.** *Top left*, reaction mechanism of the pyruvate dehydrogenase complex. Three catalytic components work sequentially, catalyzing the oxidative decarboxylation of pyruvate with the formation of acetyl-CoA, CO_2, and NADH (H^+). The reaction catalyzed by E1 is in *red*; the reaction catalyzed by E2 is in *green*; and that catalyzed by E3 is in *blue*. *Top right*, schematic representation of the domain structure of the E2ec, E2-h, and E3BP, comprising from the N-terminal end 1–3 LDs subunit-binding domain (PSBD or *S*) to which the E1 and E3 components are bound, and C-terminal catalytic domain (*C* or *C'*). *Bottom*, stepwise mechanism of PDHec reactions: *right*, tautomers and ionization states of ThDP; *left*, all kinetic constants were obtained for PDCec using methods listed in the *lower left-hand side* of the figure, and all rate constants are derived from pre-steady experiments with the exception of k_7/k_{-7} (reproduced from Ref. 73).

one lipoyl domain, the E3-binding domain, and a catalytically incompetent C-terminal domain (Fig. 1, *top right*) (4, 21–23). PDKs are recruited to the E2h of PDCh by preferentially binding to the lipoyl domains (L1, L2) of the E2h and L3 of E3BP in the E2h/E3BP core (Fig. 1, *top right*) (17, 24).

PDC of most prokaryotes, including PDCec, is the simplest form of this complex. In PDCec, 24 copies of E2ec form a cubic core through the interaction of their catalytic domains. The multiple copies of E1ec (12 E1ec dimers) and E3ec (six E3ec dimers) are bound noncovalently to the PSBD of the E2ec core, and the entire complex with mass of 4.5 MDa exhibits octahedral symmetry (25, 26).

In mammalian PDCs, two models for E2-E3BP assembly have been proposed: the "addition" model, where 60 copies of E2h and 12 copies of E3BP form the E2h-E3BP core (27), and the "substitution" model, where 48 copies of E2h and 12 copies of E3BP (28) or 40 copies of E2h and 20 copies of E3BP form the E2h-E3BP core (29). Low-resolution structural studies of E2h-E3BP and cryo-electron microscopy reconstruction of E2h-E3BP and E2h-E3BP-E3 complex support the E2h-E3BP assembly via a substitution model (30). In mammalian PDC, 20–30 heterotetramers of E1 ($\alpha_2\beta_2$) are bound to the PSBD of E2h; 6–12 homodimers of E3h are bound to the E3-binding domains (E3BD) of E3BP; and 1–3 homodimers of PDK and

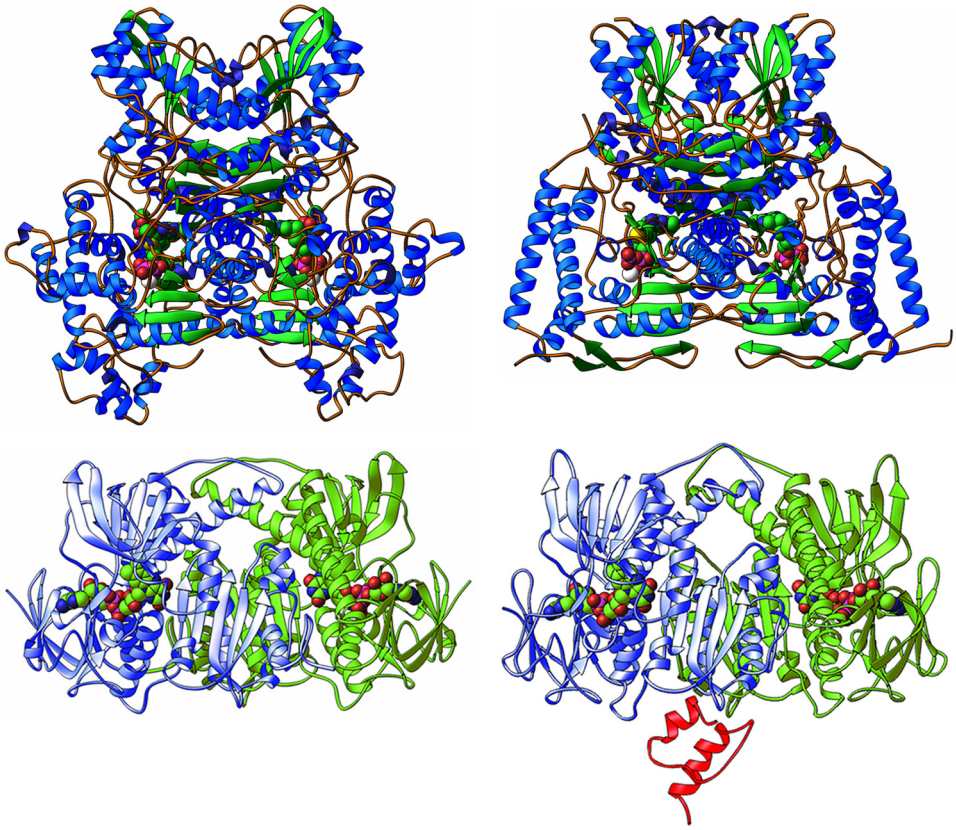

FIGURE 2. **Crystal structures of E1 and E3 from *E. coli* and human.** *Top*, ribbon diagrams illustrating structural differences between functional homodimeric and heterotetrameric E1 enzymatic assemblies from bacterial (*E. coli*), and mammalian (human) sources, respectively. *Left*, the homodimeric assembly from *E. coli* (reproduced from Ref. 34 with permission, Protein Data Bank (PDB) code 1L8A). *Right*, the heterotetrameric assembly from human (modified from Ciszak *et al.* (Ref. 31, PDB code 1NI4). The two figures are on the same scale and shown in the same orientation after least squares alignment based on the cofactors (ThDP) and structurally matching α carbons. The ThDP cofactors are shown in a space-filling representation. *Bottom*, ribbon diagrams illustrating structures of E3 functional dimers from bacterial E3ec (*left*) and human E3h (*right*) sources. In both the *left* and the *right* figures, one of the subunits is shown in *blue* and the other is shown in *green*, whereas the FAD cofactors are shown in a space-filling representation. The structure of the E3h dimer is shown in subcomplex with the E3-binding domain of E3 (in *red*). The *bottom* of this figure is reproduced from Ref. 39 for E3ec (PDB code: 4JDR) and from Ref. 33 for E3h (PDB code: 1ZY8).

2–3 heterodimers of PDP are bound to the lipoyl domains of E2h and/or E3BP. The entire complex (mass ~9 MDa) exhibits icosahedral symmetry. Hence, PDCh represents a multienzyme assembly with greater complexity in function, structure, and regulation when compared with that of PDCec.

Detailed structures of individual components of PDCh (31–35) and PDCec (36–39) have been determined by x-ray crystallography (Fig. 2). The three-dimensional structure of the E2h catalytic domain (40), a full-length E2h-E3BP core, and full-length E2-E3BP-E3 complex have been reconstructed by cryo-electron microscopy (30).

E1 Component: Structure-based Studies of the Mechanism

Catalytic Intermediates

Using recently reported high-resolution structures of several components of the PDCs and novel methods developed for deciphering different steps in the five catalytic reactions carried out by three catalytic components of the PDCec, a deeper understanding of their mechanisms has emerged in recent years. These approaches and their outcomes are summarized in this section and refer to steps in Fig. 1, *bottom*. (*a*) The first kinetic step involves the rate of deprotonation of the weak acid C2H at the thiazolium ring of ThDP to generate the reactive ylide that is measured by monitoring the rate of solvent deuterium incorporation at C2 by ^{1}H NMR (41). Both E1ec and E1h accelerate this rate substantially (42, 43). (*b*) There is evidence for the presence of three tautomeric and ionization states of enzyme-bound ThDP: the 4′-aminopyrimidine (AP form), the 1′,4′-iminopyrimidine (IP) tautomer (44–47) (Fig. 1, *bottom left*), and the protonated 4′-aminopyrimidinium (APH^+) form, detected by CD and solid state NMR (48–50). (*c*) The presence of the pre-decarboxylation intermediate C2α-lactylThDP (LThDP) could be deduced by CD as an IP tautomer (49, 51–53). An NMR method could identify ThDP-bound covalent intermediates stable under acidic conditions (LThDP, HEThDP, 2-acetylThDP) from the chemical shifts of their C6′H proton resonances (54, 55). (*d*) Existence of the first post-decarboxylation intermediate, the enamine/C2α-carbanion, could be inferred from its acid quench conversion to HEThDP and its observation by NMR (54, 55). (*e*) The second post-decarboxylation intermediate, HEThDP on enzymes also exists as the IP tautomer, as do all ThDP-bound intermediates with tetrahedral substitution at C2α atom, and could be observed by CD (52). (*f*) 2-AcetylThDP, the oxidized form of the enamine, could be an intermediate on the PDC (56, 57) and was recently observed on E1ec (derived from fluoropyruvate) via a characteristic CD signal near 390 nm (58).(*g*) Reductive acetylation of

E2 (information transfer from E1) and acetyl transfer between E2 domain are monitored by high-resolution MS methods (55), revealing that neither reductive acetylation nor interdomain acetyl transfer is rate-limiting through acetyl-CoA formation. (*h*) The formation of acetyl-CoA from [^{13}C-C2]pyruvate could be monitored via NMR methods (55), also affirming the result in step g. As shown in Fig. 1, *bottom*, all rate constants could be assessed for PDCec with these methods.

Communication between Active Center ThDPs in the E1 Component

When aligned by least squares superposition and put into a common orientation, the overall structures appear very similar, although there are great differences between the structure of the enzymes from different species (Fig. 2, *top*). In both bacterial and human E1s, there are two distinct active centers capable of binding ThDP. In the structures of E1ec (α_2 homodimer) in complex with ThDP (36) or its "transition-state analog" thiamin 2-thiothiazolone diphosphate (37), two ThDP molecules are bound at subunit-subunit interfaces forming two active centers. In the structure of E1h ($\alpha_2\beta_2$), binding of two ThDP molecules involves the opposite pair of each heterodimer (such as the diphosphate-binding domain of one $\alpha\beta$ heterodimer with aminopyrimidine-binding domain of the $\alpha'\beta'$ heterodimer and *vice versa*) with flexibility required for movement (31). No structural evidence was observed of nonequivalence of two active centers.

Steady-state kinetic and spectroscopic observations of ThDP-dependent enzymes supported communication between active center ThDPs manifested by "half-of-the site" reactivity (or alternating active-site mechanism) (42, 43, 59, 60). Solution NMR evidence of nonequivalence of two active centers in E1h and E1ec was obtained from H/D exchange kinetics at C2-H of E1-bound ThDP (42, 43) and by analysis of covalent ThDP-bound intermediates in E1ec and E1h catalysis (54, 61). A "proton wire" mechanism (62, 63) with a hydrated "tunnel" of acidic residues and water molecules was suggested to enable direct communication for proton shuttling between two ThDP N1′ atoms. Experimental evidence of such a proton wire pathway was obtained for E1ec (43). No proton wire pathway was identified for E1h.

Structural Evidence for the Role of Phosphorylation Loops in E1h

In the E1h ($\alpha_2\beta_2$), the α subunit is a target for phosphorylation/dephosphorylation by PDKs and PDPs (7, 16, 17, 64). There are three phosphorylation sites in E1h-α that could be phosphorylated independently, leading to PDC inactivation: site 1 at Ser-264-α, site 2 at Ser-271-α, and site 3 at Ser-203-α (65–68). In the E1h structure, site 1 and site 2 are located on a highly conserved phosphorylation loop A, whereas site 3 is on phosphorylation loop B (31, 33, 69). These loops are ordered on ThDP binding and disordered in its absence. On phosphorylation of site 1 (both Ser-264-α residues in the $\alpha_2\beta_2$ heterotetramer were phosphorylated), the phosphorylation loops were disordered even in the presence of ThDP, leading to loss of PDC activity (67, 69). Apparently, upon Ser-264-α phosphorylation, the bulky phosphoryl group produces a steric clash that disrupts the H-bond network involving residues from phosphorylation loop A and Tyr-33-β' from the E1p-β subunit, resulting in a disordered conformation of both phosphorylation loops (69). In E1h, the presence of both loops in the ordered conformation is a requirement for lipoyl domain recognition and substrate channeling to E2h (69). An alternative explanation based on the structure of E1h S264E (site 1 pseudo-phosphorylated) suggested that both steric and electrostatic factors affect substrate channeling between E1h and E2h (33).

The Active Center Loops in E1ec

In the structure of E1ec in complex with C2α-phosphonolactylthiamin diphosphate (PLThDP), a stable analog of the predecarboxylation intermediate LThDP, two disordered loops had become ordered and completed the active center: the inner (residues 401–413) and outer loop (residues 541–557) (38). Kinetic, spectroscopic, NMR, and structural studies demonstrated that His-407 from the inner loop and charged residues flanking His-407 have a role in stabilizing/ordering of the inner loop that is essential for substrate entry to the active site and for sequestering the active site from undesirable side reactions (38, 70–72). The E1ec variants with inner loop substitutions demonstrated greatly impaired rate of reductive acetylation of E2ec, suggesting that ordering of the inner loop plays a role in communication between the E1ec and E2ec (71, 72). For E1ec variants with E401K, H407A (inner loop), D549A (outer loop), and Y177A substitutions, the rate of LThDP formation was affected when the inner loop was disordered, also confirmed by x-ray structure of E401K and H407A E1ec variants. It was concluded that: (*a*) the rate of formation of the first C–C bond is affected when the inner loop is disordered and (*b*) loop dynamics controls covalent catalysis with ThDP (55).

Role of the E2 Component: Identification of the Interaction Loci between E1 and E2

Binding of E1h to the E1h-binding Domain of E2h

In icosahedral PDCh, the E2h-E3BP core provides the binding sites for E1h through the subunit-binding domain of the E2h. The structure of the E1 from *Bacillus stearothermophilus* (E1bs, $\alpha_2\beta_2$) in complex with the E1/E3-binding domain of E2bs (E2bs forms dodecahedral core of PDCbs) was determined (74) and was employed as a model to identify residues important for interaction between E1-binding domain of the E2h and E1h (75). It was demonstrated that the E1bs ($\alpha_2\beta_2$) binds to its cognate E2s through the C-terminal region of the β-subunits through electrostatic and hydrophobic interactions (74). Screening of the surface of the β subunit of E1h for electrostatic interactions revealed Asp-289 to be important in the formation of a salt bridge to Lys-276 on E2h (75).

Identification of the Interaction Loci of E2ec Core with E1ec and E3ec

In octahedral PDCs, the E1 binds to PSBD of E2 (PDCec) or to the E2 core domain (*E. coli* 2-ketoglutarate dehydrogenase) through their N-terminal regions. Studies of the E1ec variants with substitutions in the N-terminal region (residues 1–55) suggest that the entire N-terminal region of E1ec is responsible

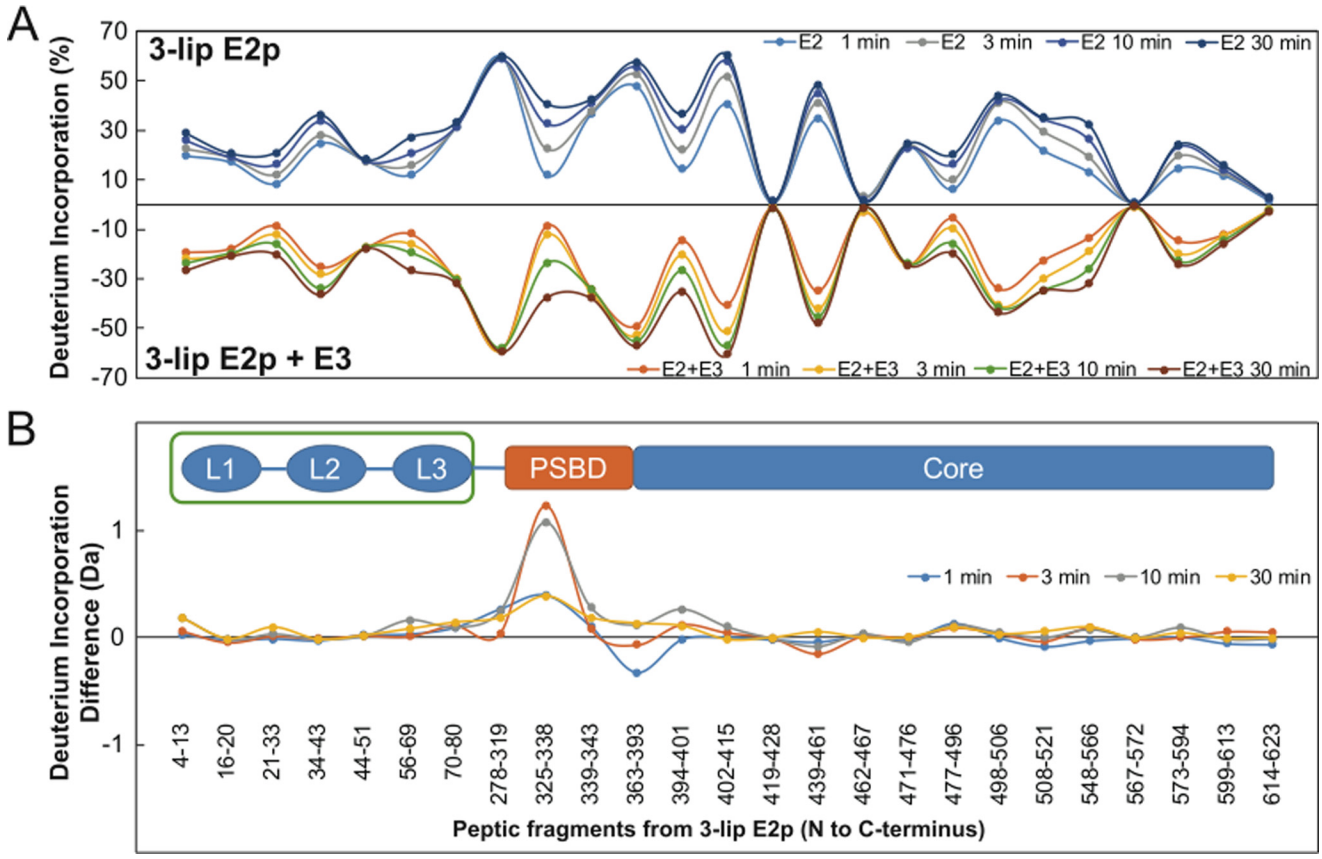

FIGURE 3. **Hydrogen deuterium exchange-MS analysis of the interaction loci of 3-lip E2ec and E3ec.** *Top figure*, butterfly plot representing average relative deuterium incorporation percentage (*y* axis) (deuterons exchanged/maximum exchangeable amides × 100) of peptic fragments from 3-lip E2ec (*x* axis, listed from N to C terminus) in the absence of E3ec (*top*) and in the presence of E3ec (*bottom*). *Bottom figure*, difference plot showing deuterium incorporation changes in peptic fragments of 3-lip E2ec in the absence and presence of E3ec (reproduced from Ref. 39).

for binding to E2ec (76, 77). Studies of E2ec variants revealed that binding of E1ec and E3ec to E2ec was strongly affected by charge-reversed substitutions at Arg-129 and Arg-150 in PSBD (numbers correspond to 1-lip E2ec sequence), but not affected by substitutions at Lys-191 and Arg-202 in the N-terminal region of the E2ec core (76). With substitutions at Arg-129 more strongly affecting the binding of E3ec and substitutions at Arg-150 strongly affecting the E1ec binding to E2ec, the studies suggested that E1ec and E3ec bind to nonidentical but overlapping loci of PSBD (76). The R129E and R150E substitutions in E2ec also affected the rate of reductive acetylation of E2ec by E1ec and pyruvate, and suggest that functional communication between E1ec and E2ec was also affected. Evidence suggests that the lipoyl domain, in addition to PSBD, is also recruited into interaction with E1ec (76). No direct interaction of the N-terminal region of E1ec with the E2ec core domain was evident.

Role of the E3 Component: Interaction loci between the E3 and E2

The functional E3 from both sources is a homodimer with two identical active centers located at the interface between two subunits (32–34, 39). Each subunit is composed of four domains: FAD-binding domain, NAD^+-binding domain, the central domain, and the interface domain. Two tightly bound FAD molecules in the E3 dimer are involved in electron transfer from dihydrolipoamide of E2 to NAD^+ with involvement of an intramolecular disulfide bridge in each subunit (Fig. 2, *bottom*). Although the overall structures are similar for E3ec and E3h, their binding mode to the E2 core is different, and will be discussed below.

Interaction Loci of E3h-binding Protein (E3BP) with E3h

The E2h-E3BP core provides the binding sites for E3h through the E3BD of E3BP. Two x-ray structures of the human E3h-E3BD subcomplex were reported (Fig. 2, *bottom*) (33, 34), revealing that E3BD binds in the interface between two E3h subunits through a combination of hydrophobic and electrostatic interactions, and complexation with E3BD did not perturb the E3 structure (33, 34). The residues involved in the E3h-E3BD interaction were identified by site-directed substitutions using kinetic, spectroscopic, calorimetric, and surface plasmon resonance analysis of the binding constants (34, 75, 78). The Ile-157 in E3BD and Tyr-438 in E3h were found important for specificity of E3h recognition by E3BD (75).

Interaction of the E2ec with E3ec Studied by Fourier Transform-Mass Spectroscopy

With the help of the known crystal structure of E3ec (39), the loci of interaction of E3ec with E2ec were identified by peptide-specific H/D exchange MS (Fig. 3). In the E2ec-E3ec subcomplex, two peptides from PSBD of 3-lip E2ec and three peptides from E3ec (two peptides from the FAD-binding domain and

one from the interface domain) exhibited significant reduction in deuterium uptake on E3ec binding to 3-lip E2ec, when compared with deuterium uptake by the individual components (Fig. 3) (39). One of the two identified peptides in 3-lip E2ec (residues 325–338) is part of the α-helix (H1), which interacts with the E3ec dimer in all known structures with the exception of the 2-oxoglutarate dehydrogenase complex (79), and it provides electrostatic stabilization of the E2ec-E3ec complex. Residue Arg-333 in this peptide (corresponding to Arg-129 in 1-lip E2ec) was identified as a "hot spot" for interaction of E2ec with both E3ec and E1ec and is a highly conserved residue in all known PSBDs (80). In summary, in PDCec, the E1ec and E3ec components bind to nonidentical, but strongly overlapping epitopes of E2ec localized in the PSBD domain. Their binding does not require competition according to the model recently suggested for differential utilization of three chains of E2ec as a trimer unit on its assembly with E1ec and E3ec (81).

Regulation of PDCh by Phosphorylation

Regulation of mammalian PDC is important for the maintenance of glucose homeostasis during both the fed and the fasted states. This control is achieved primarily by the reversible phosphorylation of E1h, resulting in its inactivation and inhibition of PDCh activity. Phosphorylation status of E1h is highly regulated by activities of PDKs and PDPs at both the transcriptional and the post-translational levels (4, 6, 7). The three sites in E1h are phosphorylated *in vivo* at different rates and with different specificity by four PDKs (66, 67). In E1h (and also in mammalian PDCs), site 1 is preferentially phosphorylated and sites 2 and 3 are sequentially phosphorylated, leading to slower rates of the complex (64). Studies of E1h variants with only a single functional site for phosphorylation (with the other two phosphorylation sites converted to alanine) revealed that each of the three sites could be phosphorylated independently, resulting in E1h inactivation in each case (66, 67). Mammalian PDK1 is capable of phosphorylating all three sites in E1h, whereas PDKs 2–4 are able to phosphorylate only sites 1 and 2. The L1 and L2 of E2h and L3 of E3BP play an essential role in regulation of PDK activities (Table 1). Again, different binding preferences of four kinases toward the three lipoyl domains (L1, L2, and L3) provide flexibility for modulation of their activities. Binding of PDKs to the lipoyl domains of E2h and E3BP co-localizes to its substrate, E1h, bound to E2h, and also induces conformational changes in PDKs, resulting in variable degrees of activation, with PDK3 showing the highest degree of activation (Table 1). Interestingly, reduction by NADH and acetylation by acetyl-CoA of the lipoyl moiety result in variable degrees of stimulation of PDKs, with PDK2 being the most sensitive to this stimulation (Table 1). In contrast, high levels of pyruvate, ADP, NAD^+, and CoA are inhibitory for PDK activities (16). The activity of PDC is tightly regulated by the cellular levels of PDKs and PDPs in tissues under different nutritional and disease states (7). Transcriptional modulation of these regulatory enzymes is under the influence of hormonal changes during starvation, diabetes, and hyperthyroidism (6, 7). PDK4 expression is regulated by fatty acids through peroxisome proliferator-activated receptor α (47) and also by glucocorticoids (48). Detailed accounts of transcriptional regulation of PDKs can be found in a recent comprehensive review (82, 83). Detailed aspects of transcriptional regulation of PDKs can be found in a recent, comprehensive review (11). Recent findings suggest a role of post-translational modifications in regulation of PDCh, including activation of PDK1 by tyrosine phosphorylation (85) and inhibition of PDP1 and E1h by lysine acetylation (86), that control PDC activity in cancer cells. These issues need more detailed elucidation.

TABLE 1
Characteristics of mammalian PDK isoenzymes and PDP isoenzymes
The information presented in Table 1 is derived from Refs. 3, 6, 7, 11, 16, 24, 64, 65, 86, and 87.

Properties	PDK1	PDK2	PDK3	PDK4	PDP1	PDP2
Tissue Distribution[a]	H, SM, L, P	H, SM, L, K, B, P, T, Lg, S	T, Lg, B, K, H	SM, H, Lg, L, K	H, B, T, Sk, Lg	L, K, H, B, S
Binding to LDs	L1, L2	L2 > L1	L2 > L1	L2 > L1	L2	
Activities of PDK and PDP (munits/mg of protein)						
site 1	63	125	36	70	72	23
site 2	20	32	74	41	322	47
site 3	24	0	0	0	80	32
The extent of PDKs activation on binding to LDs (fold)						
site 1	1	1.7	6.6	1		
site 2	3.6	6.3	29.0	1.7		
site 3	3.2	-	-	-		
The extent of PDKs activation on reduction and acetylation of LDs (fold)						
	1.5	2.4	1.2	2.0		
Effectors of PDKs, PDPs activities[b]	(+) NADH (+) AcCoA (-) ADP (-) Pyruvate	(+) NADH (+) AcCoA (-) ADP (-)Pyruvate	(-)Pyruvate (weak)	(+) NADH (+) AcCoA (-) ADP (-) Pyruvate	(+) Mg^{2+} (+) Ca^{2+}	(+) Spermine

[a] H, heart; SM, skeletal muscles; L, liver; Lg, lungs; K, kidneys; B, brain; P, pancreas; T, testis; S, spleen.
[b] (+) activation; (−) inhibition.

The crystal structures of all four PDKs (either alone or in association with L2, ADP, or ATP) have been reported (87–93). Each PDK monomer has two domains of about equal size, the N-terminal domain and the C-terminal domain, and these two domains are connected by a flexible, poorly ordered loop. In dimeric PDK, the C-terminal tail of each monomer interacts with the lipoyl-binding pocket in the N-terminal domain of the other monomer. Monomers in the dimeric PDK are in a head-to-head orientation with the primary interaction between the C-terminal domains. In the absence of L2, PDK dimer forms a "closed" conformation, resulting in closing of the active-site cleft because of the disordered C-terminal tail of one monomer not interacting with the lipoyl-binding pocket of the opposite monomer (91–93). This conformation stabilizes the ATP lid and thus prevents dissociation of ADP, resulting in product inhibition. Upon binding of PDK3 to L2, the crossover configuration of the C-terminal tails results in an "open" conformation promoting the widening of the active-site cleft, causing disordering of the ATP lid and accelerating the release of trapped ADP (88, 89, 91). The two lipoyl-binding pockets are located on the outer surface of the PDK dimer in the opposite direction, suggesting that PDK may bind to two different lipoyl domains on PDC at a time. This arrangement is consistent with a "hand-on-hand" movement of PDK to serve many E1s in the complex (16).

PDP exists as two different forms, PDP1 (catalytic and regulatory subunits) and PDP2 (catalytic subunit only), with differ-

ing biochemical characteristics. PDP1c (catalytic) activity strongly depends on its binding to the lipoyl domain of E2h requiring Ca^{2+} (24, 94–96). In contrast, PDP2c requires neither for its activity (95). Both PDP1c and PDP2c are able to dephosphorylate all three phosphorylation sites on E1h. PDP1c exhibits a random mechanism of dephosphorylation (with relative rates of site 2 > site 3 > site 1), indicating a lack of site-site dependence for dephosphorylation. In contrast, PDP2c displays interdependence in dephosphorylation of site 1 with site 2 and site 3 separately, and no interaction between site 2 and site 3 (95). The crystal structure of PDP1c reveals a unique hydrophobic pocket on the surface to accommodate the lipoyl moiety of the lipoyl domain of E2 (97). It is proposed that the closure of the lipoyl moiety-binding site is achieved by the formation of the intermolecular (PDP1c/L2) Ca^{2+}-binding site.

Concluding Remarks

More than 60 years have passed since lipoic acid was first isolated and characterized by Reed *et al*. (84), a seminal discovery in our understanding of the chemistry and core functions of these complexes. We now have x-ray structures of several PDC components. With advances in detecting ThDP-bound intermediates using spectroscopic methods, the rates of individual steps could be determined for the *E. coli* PDC, revealing that formation of the first covalent complex between pyruvate and ThDP is rate-limiting, and this step may be controlled by the mobility of active center loops. Remaining challenges include the need for a high-resolution structure of an intact E2 component, and of course of the entire complex. Renewed interest and enthusiasm for studying the human PDC are warranted by its recently revealed involvement in some forms of cancer, type 2 diabetes, and obesity, probably involving the interactions of the E2h-PDK subcomplex and providing new targets for rational drug design to regulate glucose metabolism in cancer, type 2 diabetes, obesity, and other diseases.

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