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Sidechain Biology and the Immunogenicity of PDC-E2, the Major Autoantigen of Primary Biliary Cirrhosis

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Abstract

The E2 component of mitochondrial pyruvate dehydrogenase complex (PDC-E2) is the immunodominant autoantigen of primary biliary cirrhosis. Whereas lipoylation of PDC-E2 is essential for enzymatic activity and predominates under normal conditions, other biochemical systems exist that also target the lysine residue, including acylation of fatty acids or xenobiotics and ubiquitinylation. More importantly, the immunogenicity can be affected by derivatization of the lysine residue, as the recognition of lipoylated PDC-E2 by patient autoantibodies is enhanced compared with octanoylated PDC-E2. Furthermore, our laboratory has shown that various xenobiotic modifications of a peptide representing the immunodominant region of PDC-E2 are immunoreactive against patient sera. The only purported regulatory system that prevents the accumulation of potentially autoreactive PDC-E2 is glutathionylation, in which the lysine-lipoic acid moiety is further modified with glutathione during apoptosis. Interestingly, this system is found in several cell lines, including HeLa, Jurkat, and Caco-2 cells, but not in cholangiocytes and salivary gland epithelial cells, both of which are targets for destruction in primary biliary cirrhosis. Hence, the failure of this or other regulatory system(s) may overwhelm the immune system with immunogenic PDC-E2 that can initiate the breakdown of tolerance in a genetically susceptible individual. In this review the authors survey the data available on the biochemical life of PDC-E2, with particular emphasis on the lysine residue and its known interactions with machinery involved in various posttranslational modifications.

Primary biliary cirrhosis (PBC) is an archetypical organ-specific autoimmune disease that predominantly affects middle-aged women and includes a chronic and progressive inflammatory response, leading to destruction of intrahepatic bile duct epithelial cells (cholangiocytes). The diagnostic hallmark of PBC is the antimitochondrial antibody (AMA) that reacts specifically with E2 components of 2-oxoacid dehydrogenase enzymes, particularly the 74-kd E2 subunit of the pyruvate dehydrogenase complex (PDC-E2); 90% to 95% of patients with PBC have high titer AMAs long before onset of clinical symptoms.^{1,2} We believe that a thorough understanding of the biochemical nature of enzymatic interactions of PDC-E2 is key to unraveling the enigma of PBC.

Pyruvate Dehydrogenase Complex

PDC is a non-covalent enzymatic complex that catalyzes the oxidative decarboxylation of pyruvate to acetyl CoA. The complex consists of a multimer core of dihydrolipoyl transacetylase (E2) chains arranged with icosahedral symmetry on which the other protein components are arranged.³ The role of E2 to shuttle an acetyl group to CoA to produce acetyl CoA is supported by the structure of the protein wherein the lysine side-chain derivatized by the lipoic acid is mobile and exposed to solvent. PDC is a critical enzyme in the control of intermediate metabolism because it modulates carbohydrate metabolism by regulating the entrance of carbohydrate-derived acetyl units into the tricarboxylic acid cycle. As such, it is under a complex series of controls that affect its activity both acutely as well as long term.

All of the subunits of PDC are nuclear encoded, with each newly synthesized polypeptide containing an amino-terminal extension, termed a presequence, that guides the post-translational transport of preproteins to the mitochondria (Fig. 1).⁴ Bound cytosolic HSP70 (heat shock protein) prevents preproteins from spontaneous aggregation or folding before they associate with a presequence receptor on the mitochondrial outer membrane. Subsequently, cytosolic HSP70 is liberated, and, concomitantly, preproteins transverse the outer and inner membranes through translocase complexes. As soon as the preprotein emerges from the translocase of the inner membrane, mitochondrial HSP70 binds to the imported protein to pull the unfolded protein into the matrix. Mitochondrial peptidases then cleave the presequence, allowing the subunits to autoassemble into an apo-PDC (*i.e.*, nonfunctional). Subsequently, lipoic acid is covalently attached to the E2 subunit conferring enzymatic activity to PDC.

The limited studies that have examined long-term regulation of PDC levels have shown that high sucrose feeding increases total PDC activity in liver but not in heart and skeletal muscle.^{5,6} Conversely, aerobic training alters total PDC activity in skeletal muscle.⁷ Interestingly, both the sucrose feeding and aerobic exercise-mediated increases in total PDC appear to be driven solely by changes in E1 α subunit expression, whereas expression of the other PDC subunits remains constitutive. These differences in regulation between the different PDC subunits suggest that other mechanisms may be involved in avoiding a mismatch between E1 α levels and those of E2 and other PDC components. Finally, the demonstration of tissue-specific regulation of the expression of a ubiquitous metabolic enzyme such as PDC raises issues as to how tissue specificity is achieved.

Mitochondrial Autoantigens

A paradox in PBC is that mitochondrial proteins are present in all nucleated cells, yet the autoimmune attack is directed with high specificity to the biliary epithelium. The central conundrum regarding PBC is that, although investigators during the past several decades have extensively mapped the nature of the immune response in terms of its B- and T-cell epitopes, this has not led to progress in understanding the cause of disease. Little emphasis has been placed on how these proteins are modified endogenously or indirectly through external influences. Three recent findings suggest that this area may be of considerable importance in understanding PBC. One is the demonstration that the redox state of cells, and more specifically, whether the lysine-lipoyl moiety of the E2 protein was derivatized with glutathione during apoptosis, determines whether PDC-E2 can be recognized by autoantibodies.⁸ Second is the fact that epithelial cells handle PDC-E2 in a manner different from that of other cells of the body. That is, they do not attach a glutathione to the lysine-lipoyl group during apoptosis. Finally, specific xenobiotic modifications of the inner lysine-

lipoyl domain of PDC-E2 have been shown to be immunoreactive when probed with patient sera,^{9–12} again suggesting the status of the lysine-lipoyl moiety is important.

Whereas PDC-E2 is the major autoantigen in PBC, other members of the 2-oxoacid dehydrogenase complexes are targets of AMA, including the E2 subunit of the branched chain 2-oxoacid dehydrogenase complex, the E2 subunit of the 2-oxoglutarate dehydrogenase complex, and as well as the E3BP subunit of PDC.^{13–19} The nature of the E2 chains of PDC, the E2 subunit of the branched chain 2-oxoacid dehydrogenase complex, and the 2-oxoglutarate dehydrogenase complex is such that they all share a common protein motif that consists of a N-terminal domain with lysine-lipoyl group(s), a peripheral subunit binding domain, and a C-terminal inner core responsible for acyltransferase catalytic activity.²⁰ Whereas the E3BP subunit is distinct from E2 subunits, it too exhibits this motif, as it has a N-terminal region of lysine-lipoyl domains, a subunit binding domain, and an inner core domain.^{21–24}

Very extensive studies have mapped the autoepitopes recognized by B cells through the use of recombinant proteins, truncated constructs, and a combination of peptides. These studies have shown that the immune reactivity of AMA is directed against a conformational epitope that includes a lysine-lipoyl domain.^{25–28} These domains contain signature motifs of amino acids ETDKA, ET-DK(T), and (QS)DKA wherein the lipoic acid is covalently attached to the ϵ group of lysine (K) via an amide bond (Table 1). Whereas all four mitochondrial autoantigens contain at least one lysine-lipoyl acid domain, PDC-E2 has two (inner and outer) lysine-lipoyl domains. Of note, although AMAs react with the outer lysine-lipoyl domain (amino acid sequence 1-90) of PDC-E2, they do so at a significantly lower (100-fold) titer. This implies that the predominant epitope lies within the inner lysine-lipoyl domain, specifically at residues 128 to 221 (93 amino acids).^{16,25} Interestingly, the AMA response to lipoylated recombinant PDC-E2 is of substantially higher titer and affinity than to the unlipoylated antigen,²⁹ suggesting that lysine-lipoate may be a part of the immunodominant epitope.

Subsequent T-cell epitope mapping of PDC-E2 has shown that the minimal epitope required for a CD4 T-cell response maps to the amino acid sequence 163-176 (GDLLEIETDKATI) of the inner lysine-lipoyl domain, as well as to the outer lysine-lipoyl domain (amino acid sequence 36-49; GDLIAEVETDKATV).^{30,31} In addition, our laboratory has identified a class I-restricted CD8 T-cell epitope for PDC-E2 that was recognized by CD8 T cells derived from peripheral blood and liver of patients with PBC.³² Interestingly, this peptide is located at residues 159 through 167, which overlap the class II-restricted CD4 T-cell epitope (amino acids 163-176).³² Note that the peptides used to elicit T-cell responses in these studies are unlipoylated at the lysine residue. Hence, it would be interesting to examine the effects of posttranslationally modified peptides on the stimulation of T cells. Because lipoylation of PDC-E2 enhanced its recognition by AMAs, it is plausible that lipoic acid modification of the T-cell epitopes can increase the binding affinity of peptide to major histocompatibility complex (MHC) molecules, which can augment T-cell responses. Future studies regarding the three-dimensional analysis of the interaction of lysine-derivatized PDC-E2 peptide with MHC molecules should be performed.

The clustering of immunodominant T-cell and B-cell epitopes of PDC-E2 is not unusual; a similar theme is observed with autoantigens in other autoimmune diseases, including multiple sclerosis^{33,34} and type 1 diabetes mellitus.^{35,36} According to conventional antigen presentation pathways, CD4 T cells recognize epitopes derived from exogenous proteins, whereas CD8 T cells are stimulated by endogenously derived antigens. Therefore, the antigen sources for the induction of CD4 and CD8 T cells are theoretically generated through different pathways and may not even be derived from the same polypeptide

molecule to explain the clustering of T-cell and B-cell epitopes. However, recent work from the Gershwin lab suggests that exogenous PDC-E2, possibly derived from apoptotic cellular turnover, may be presented to both CD4 and CD8 T cells. Kita et al.³² have shown that the induction of CD8 T cells is augmented by PDC-E2–autoantibody immune complexes cross-presented by dendritic cells. Such autoantibodies that are bound to the immunodominant B-cell epitope also will encompass the CD4 and CD8 T-cell epitopes and protect them from degradation by proteases once the immune complex is efficiently internalized by dendritic cells. In this situation, the immunodominant CD4 T-cell epitope may be presented by conventional MHC class II pathways to CD4 T cells, whereas the CD8 epitope may be presented by MHC class I through cross-presentation.³⁷

Lipoylation

The B-cell and T-cell epitopes seen by AMA and T cells all recognize peptide sequences that contain a lysine-lipoyl residue. More generally, lysine as an amino acid can serve as a modification site by a variety of posttranslational protein processing systems. These protein modifications cover a gamut of reactions involving lysines reacting with a range of compounds to become ubiquitinated or acylated, with lipoylation in PDC-E2 being a specific example. However, despite the seemingly critical nature of lipoic acid as a cofactor in a wide range of biochemical processes, lipoic acid metabolism in mammalian cells remains incompletely characterized. Recent work has shed significant light on the steps involved in the incorporation of lipoic acid into proteins. A murine gene encoding lipoic acid synthase, designated mLIP1, has been identified³⁸ and is functionally homologous to *Escherichia coli* LipA. The protein product catalyzes the insertion of sulfur atoms into octanoic acid endogenously produced from mitochondrial fatty acid biosynthesis and added to the lysine of PDC-E2, for example (Fig. 2). Thus, mLIP1 serves to produce endogenous lipoic acid via *de novo* synthesis using this fatty acid, sulfur, and S-adenosyl methionine.³⁹ The level of mLIP1 activity is unclear, but its existence holds out the possibility for bypassing the more complex system of generating lipoylated proteins (see later discussion). In addition, it is believed that humans can efficiently scavenge lipoic acid provided by dietary intake via intestinal vitamin transporters.⁴⁰

In mammals, the posttranslational modification of unlipoylated apoproteins by addition of lipoic acid is done in a two-step enzymatic reaction using free lipoic acid. The free lipoic acid in the cell is first activated by reacting it with adenosine triphosphate, using up two high energy phosphate bonds to form lipoyl-AMP (GMP), a process catalyzed by lipoate activating enzyme (LAE).⁴¹ The activated lipoyl moiety is subsequently used to form an amide bond with lysine residues of the unlipoylated apoprotein, which is catalyzed by lipoyl-AMP(GMP):N-lysine lipoyl transferase (Fig. 3).^{42,43}

Interestingly, purified LAE from bovine liver mitochondria preferentially utilizes guanosine triphosphate, instead of adenosine triphosphate, for the activation of lipoic acid to form lipoyl-GMP.⁴⁴ Furthermore, genetic analysis of LAE reveals that the enzyme is identical to xenobiotic-metabolizing/medium-chain fatty acid:CoA ligase-III (XL-III) (also from bovine liver mitochondria).⁴⁴ Hence, there appear to be two pathways in which LAE is capable of activating carboxylic acids: one that uses guanosine triphosphate to form acyl-GMP; the other is dependent on adenosine triphosphate for the activation of carboxylic acids to the CoA thioester (acyl-CoA) via an acyl-AMP intermediate. It is particularly important to note the broad substrate specificity exhibited by these activating enzymes, which suggests that a variety of activated carboxylic acids, not just lipoic acid, may be available to be conjugated to lysine residues. Apparently, the mitochondrial matrix contains several other broad specificity ligases with activity toward medium-chain fatty acids and a wide range of xenobiotic carboxylic acids,^{45–49} perhaps indicating that acylation of lipoyl domains by

xenobiotics may not be an uncommon event. These enzymes, including LAE and XL-III, are collectively referred as xenobiotic/medium-chain fatty acid: CoA ligases (XM-ligases)⁴⁹; recently, the human homologue of XM-ligase, termed HXM-A, has been cloned from liver mitochondria.^{50,51}

Bovine lipoyl transferase is equally efficient at transferring activated acyl groups from lipoate and other fatty acid nucleosides (hexanoyl-, octanoyl-, and decanoyl-AMP) to the lysine-lipoyl domains of apoproteins, including PDC-E2.⁴² Depending on the availability of lipoic acid, lipoyl transferase can transfer octanoyl moiety from octanoyl-AMP to apoproteins when lipoyl-AMP is limiting. Indeed, it has been shown that octanoylated PDC-E2 are recognized by AMA, but not as effectively as their lipoylated counterparts.⁵²

One potential hypothesis regarding PBC is that xenobiotic exposure may alter self-proteins enough to break tolerance. The Gershwin lab has systematically modified a peptide encompassing the inner lysine-lipoyl domain of PDC-E2 with 18 different xenobiotic structures conjugated to the lysine residue in place of lipoic acid.⁹ Significantly, sera from PBC patients were shown to react against three of these xenobiotic modified autoepitopes significantly better than to the native domain.⁹ Subsequently, immunization of rabbits with one of these compounds, 6-bromohexanoate, induced AMA with reactivity against PDC-E2.¹¹ Although this model recognized the breakdown of tolerance at the serological level, histopathological features characteristic of PBC observed in humans were not reproduced. Such failure to produce PBC-like lesions within the liver lend support to the common notion that PBC is a complex disease that requires a genetic predisposition for pathogenesis. Moreover, the evaluation of rabbit T cell responses to 6-bromohexanoate was not pursued in this study. Nevertheless, existing data on the serological recognition of xenobiotics warrant further studies examining the capacity of xenobiotic-modified PDC-E2 to induce T-cell activation in humans.

Ubiquitinylation

Another protein processing system that uses a lysine residue as a target is ubiquitinylation, which represents a key posttranslational modification event for a variety of intracellular proteins.⁵³ Although it is clear that ubiquitinylation targets aberrant proteins for degradation, the mechanism(s) that mediate the cellular decision to recognize malformed polypeptides and abort folding attempts remain uncharacterized. However, accumulating evidence has suggested that a molecular chaperone, identified as a carboxyl-terminus of HSC70 (heat shock cognate protein 70) interacting protein (CHIP),⁵⁴ is critical in regulating the cellular balance between folding and degradation.⁵⁵ CHIP contains two distinct domains: (1) the tetratricopeptide repeat domain that associates with members of heat shock proteins, including HSP70, HSC70, and HSP90^{54,56,57}; and (2) a U-box domain that possesses intrinsic E3 ubiquitin ligase and facilitates protein polyubiquitination.⁵⁸ Hence, CHIP provides a direct link between the chaperone (folding) system and the ubiquitin–proteasome (degradation) system. Recently, Meacham and colleagues demonstrated that CHIP cooperates with HSC70 to target aberrant forms of cystic fibrosis transmembrane-conductance regulators for proteosomal degradation by promoting their ubiquitinylation.⁵⁷ The use of a lysine residue for the attachment of ubiquitin raises the potential for competition between glutathionylation and ubiquitinylation. The multiple lines of evidence pointing to the apparent centrality of the lysine lipoate in PBC suggest that this relationship(s) needs to be characterized to understand the nature of the lysine PBC link.

Glutathionylation: Masking of PDC-E2

One potential route for the release of PDC-E2 might be during apoptosis, the process of controlled cellular death. Although investigations regarding the potential roles of apoptosis

in PBC are limited, the most significant finding is that apoptosis initiates a special sequence of events such that nonepithelial cells mask their E2 lysine-lipoyl group. This concept of the need to prevent the accumulation and subsequent exposure of these potentially self-reactive antigens to the immune system is strongly supported by several different lines of research. One line of research has shown that AMA appeared to preferentially recognize PDC-E2 with reduced sulfhydryl groups,⁵⁹ suggesting that blocking these sites would likely eliminate the epitope site responsible for the generation of autoantibodies. This has been followed up by studies directed at characterizing the autoantibody recognition site and the effects of apoptosis on the immunogenicity of PDC-E2.⁸ The authors showed that, after apoptosis, HeLa, Jurkat T, and Caco-2 cells presented a form of PDC-E2 that becomes undetectable when probed with AMA. This loss of recognition of apoptotic cell-derived PDC-E2 by AMA is not attributable to disappearance or degradation, but rather to a reversible structural change in the protein from which the epitope can be recovered. However, most notably, autoantibody recognition of PDC-E2 persists in apoptotic rat cholangiocytes, as well as human salivary gland epithelial cells. This immunoreactivity can be eliminated with the addition of oxidized glutathione to sodium dodecyl sulfate-treated cholangiocyte cell lysates that render PDC-E2 nonantigenic when probed with AMA. Conversely, apoptotic HeLa cells pretreated with buthionine sulfoximine (a glutathione synthetase inhibitor) before apoptotic stimulus were unable to prevent the recognition by patient sera, thereby “resurrecting” the antigen with the depletion of glutathione. The authors therefore concluded that the persistent recognition of PDC-E2 derived from cholangiocytes and human salivary glands epithelial cells is caused by a failure in these cells to covalently link PDC-E2 to glutathione during the course of apoptosis (Fig. 4). These data indicate that the epithelial cells most frequently affected in patients with PBC are unique, in that they do not block or mask the immunoreactivity of AMA.

A puzzling finding is that when HeLa cells were transfected with Bcl-2, they too retain AMA reactivity to PDC-E2, mimicking what is seen in cholangiocytes. Decreased levels of Bcl-2, an antiapoptotic protein with antioxidant properties,⁶⁰ is associated with glutathione depletion and increased apoptosis in cultured cholangiocytes.⁶¹ Human and normal rat cholangiocytes are unique in that they constitutively express high levels of Bcl-2,^{62–64} which would also correlate with elevated glutathione levels. Therefore, it would seem contradictory to find an apparent lack of glutathionylation in these cells that demonstrated high levels of Bcl-2.

The immunologically significant masking of PDC-E2 requires sulfhydryl groups for the incorporation of glutathione. The reduced form of glutathione can directly bind with –SH groups contained in a polypeptide after their oxidation, forming so-called “mixed” (protein–nonprotein) disulfide bridges (protein-SSG).⁶⁵ However, in the presence of increased cell levels of oxidized glutathione, protein glutathionylation can occur via thiol/disulfide exchange reactions.⁶⁵ Moreover, protein glutathionylation is a reversible process, and deglutathionylation of protein-SSG is primarily catalyzed by glutaredoxins.⁶⁶ Importantly, protein glutathionylation-deglutathionylation is a dynamic process under physiological conditions and reflects the redox state of the –SH in the cell.⁶⁵ Perhaps, cholangiocytes exhibit increased activity of glutaredoxins during apoptosis, which would reconcile the apparent paradox of Bcl-2–dependent release of unmasked PDC-E2. Moreover, the sequences of the inner and outer lysine-lipoyl domains of PDC-E2 contain no cysteine or a sulfhydryl group, unless lipoic acid has been conjugated to the lysine. Because lipoic acid enhances the antigenicity of PDC-E2, it is tempting to postulate that glutathionylation of E2 proteins during apoptosis provides a protective mechanism whereby many cell types block the release of potentially pathogenic autoepitopes.

The use of glutathionylation as a critical processing event in determining recognition by AMA suggests that although lipoylation of the lysines in PDC-E2 is required for enzymatic activity and predominates under normal conditions, the tradeoff is exposing the immune system to a more immunogenic antigen than the unlipoylated or octanoylated PDC-E2. Therefore, it appears that proteins containing lysine-lipoate must be highly regulated to prevent the inappropriate release of these proteins. In addition, one feature of these proteins is their multimeric nature. In fact, the PDC complex can be used to provide a platform for the presentation of multiple copies of an antigen. Hence, these complexes form a scaffold for multiple epitope display that elicits both a cellular and a humoral immune response.⁶⁷ Very recent papers have shown that a large number of proteins, including HSP70, ubiquitin, and ubiquitinylation-related proteins, are targets for glutathionylation.^{68,69}

Conclusion

In PBC, the autoreactivity against PDC-E2 is such that the B-cell, the CD4, and the CD8 T-cell responses are all directed at regions of PDC-E2 that contain a lysine, the specific amino acid targeted for addition of lipoic acid. However, the paradoxical nature of PBC is that, although the autoimmune attack is directed against ubiquitous mitochondrial autoantigens, the pathology is focused toward the biliary epithelial cells lining the bile ducts. In addition to lipoylation, accumulating data highlighting posttranslational modifications of PDC-E2 suggest that biochemical systems compete for targeted lysine residues, including acylation of fatty acids or xenobiotic, and ubiquitinylation. Any of these would then block the lysine and, because of the absence of a sulphhydryl, render the E2 epitope unmaskable by glutathione. Another potential mishandling of the lysine-lipoate of E2 occurs when cellular redox status/reduced form of glutathione availability are unable to support either glutathionylation or caspase cleavage of the PDC-E2 epitope. Thus, cellular stress in susceptible individuals might result in the failure to modulate lysine-derivatized PDC-E2 and result in the release of immunogenic antigens. This would then impose a burden on the immune system to prevent a runaway immune response to these molecules. These facts suggest that PBC requires the intersection of the excessive release of immunologically active PDC-E2 in a host whose immune system does not adequately damp reactions involving self-antigens.

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Abbreviations

PBC	primary biliary cirrhosis
AMA	antimitochondrial autoantibody
PDC-E2	E2 subunit of pyruvate dehydrogenase complex
HSP70	heat shock protein 70
MHC	major histocompatibility complex
LAE	lipoate-activating enzyme
LT	lipoyl transferase
CHIP	carboxyl-terminus of HSC70 interacting protein

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Fig. 1. Schematic representation of the post-translational import of PDC subunits into the mitochondria. cHSP70, cytosolic heat shock protein 70; mtHSP70, mitochondrial heat shock protein 70.

Fig. 2.

Endogenous synthesis of lipoic acid from octanoate. In mammalian cells, this reaction is catalyzed by a lipoic acid synthase, termed mLIPI.

Fig. 3.

Possible acylated derivatives of PDC-E2 in mammalian systems. **Solid arrows** represent established processes, while the **dashed arrow** remains to be determined. Enzymes are shown in italic. XB, xenobiotic; LAE, lipoate activating enzyme; LT, lipoyl-AMP(GMP): N^{ϵ} -lysine lipoyltransferase. *Other fatty acids may be substituted for octanoic acid.

Fig. 4.
The role of the glutathionylation system in the immunogenicity of PDC-E2.

Table 1

Amino Acid Sequence of Flanking Lipoyl-Lysine Residues in the Four Mitochondrial Proteins Containing the Lipoic Acid Binding Domain

Autoantigen	Amino Acid Sequences
PDC-E2 (inner lipoyl domain)	G D L L A E I E T D K [*] A T I
PDC-E2 (outer lipoyl domain)	G D L I A E V E T D K [*] A T V
E3BP	G D A L C E I E T D K [*] A V V
OGDC-E2	D E V V K E I E T D K [*] T S V
BCOADC-E2	F D S O C E V Q S D K [*] A S V

* Specific lysine residue where lipoic acid is attached via an amide bond.

Abbreviations: PDC-E2, E2 subunit of pyruvate dehydrogenase complex; E3BP, dihydrolipoamide dehydrogenase-binding protein; OGDC-E2, E2 subunit of the 2-oxoglutarate dehydrogenase complex; BCOADC-E2, E2 subunit of branched chain 2-oxoacid dehydrogenase complex.