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REVIEW ARTICLE

The 2-oxo acid dehydrogenase complexes: recent advances

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INTRODUCTION

The 2-oxo acid dehydrogenase complexes represent the classic examples of multienzyme complexes, a knowledge of whose structure and function has wide implications for our understanding of macromolecular assembly and organization and of protein structure and function. Each complex consists of multiple copies of three enzymes, termed E1, E2 and E3. E1 is a 2-oxo acid dehydrogenase which has thiamin pyrophosphate as an essential co-factor. E2 is a dihydrolipoamide acyltransferase, with a covalently attached lipoic acid cofactor, and E3 is an FAD-containing dihydrolipoamide dehydrogenase. The components catalyse consecutive steps in the overall reaction, as outlined in Fig. 1. These complexes have molecular masses of several million [mammalian pyruvate dehydrogenase complex (PDC) is slightly larger than a ribosome] and are truly self-assembling [1,2].

These macromolecules are not however merely of interest from a structural viewpoint. Each of the 2-oxo acid dehydrogenase complexes occupies key positions in intermediary metabolism and the activity of each mammalian complex, located within mitochondria, is under stringent control by hormones and dietary factors. PDC oxidatively decarboxylates pyruvate to acetyl-CoA, an irreversible step in the utilization of carbohydrate, whilst 2-oxoglutarate dehydrogenase complex (OGDC) is a potentially regulatory enzyme of the tricarboxylic acid cycle. Branched-chain 2-oxo acid dehydrogenase complex (BCOADC) catalyses an irreversible step in the catabolism of several essential amino acids, including the branched-chain amino acids [2]. PDC and OGDC have narrow substrate specificities, with OGDC being essentially specific for 2-oxoglutarate, whereas PDC will oxidize 2-oxobutyrate, in addition to pyruvate, at a significant rate [3]. In contrast, BCOADC has a relatively broad specificity, oxidizing 4-methylthio-2-oxobutyrate and 2-oxobutyrate at comparable rates and with similar K_m values as for the three branched-chain 2-oxo acid substrates, namely 3-methyl-2-oxobutyrate, 4-methyl-2-oxopentanoate and 3-methyl-2-oxopentanoate [3–5]. BCOADC will also oxidize pyruvate, but the K_m value is approx. 1 mM (as compared with 30 μM with PDC [4]), making this unlikely to be of physiological significance [5].

Structural analysis of the complexes

The powerful combination of protein chemistry and molecular genetics has led to elucidation of the structure and function of the complexes. Much of the structural analysis has been carried out using complexes of bacterial origin, although recently significant progress has been made with the mammalian complexes.

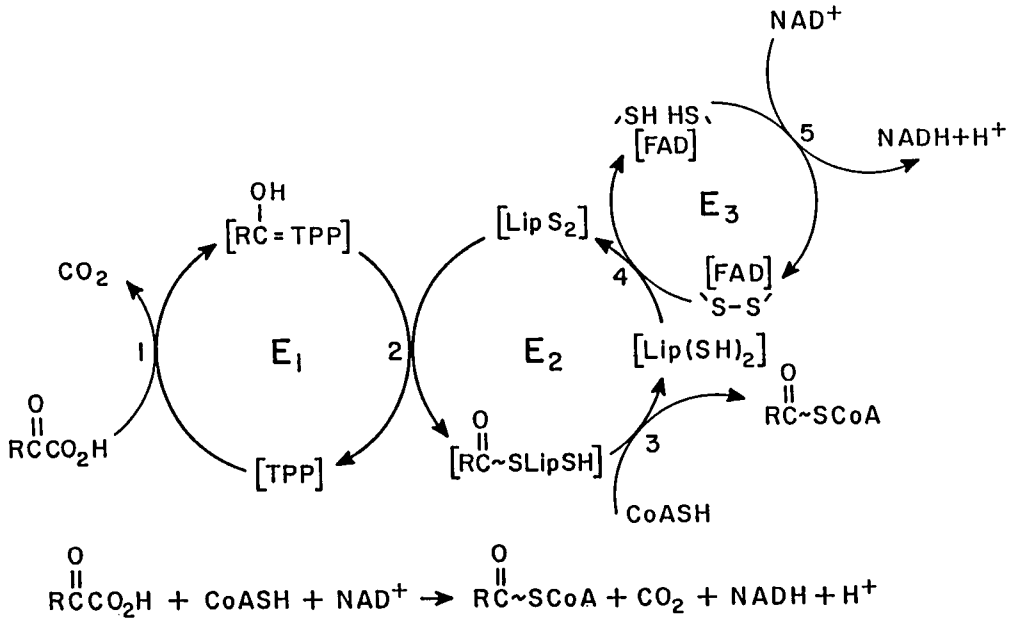

Fig. 1. Reaction scheme for the oxidative decarboxylation of the 2-oxo acids by the respective 2-oxo acid dehydrogenase complexes

E1 catalyses both reactions 1 and 2.

Abbreviations used: PDC, pyruvate dehydrogenase complex; OGDC, 2-oxoglutarate dehydrogenase complex; BCOADC, branched-chain 2-oxo acid dehydrogenase complex.

The E2 component of the respective complexes is the key to their structure and function. It serves three major roles: (1) it forms a central, symmetrical core around which are arranged multiple copies of the E1 and E3 components, (2) it is an acyltransferase, catalysing the formation of the acyl-CoA product, and (3) it provides the attachment site for the lipoic acid cofactor which interacts with the different active sites in the complexes. It has been shown recently by ^{1}H-n.m.r. that acetylation occurs at the 8-S position of the lipoic acid and not at the 6-S position, although isomerization can occur in aqueous solution [6].

The E2 components of the complexes are arranged with point group symmetry. OGDC and BCOADC have 24 copies of E2 arranged with 432 (octahedral) symmetry as does PDC from Gram-negative bacteria, whilst PDC from mammals, birds, yeast and Gram-positive bacteria has 60 copies of E2 arranged with 532 (icosahedral) symmetry [7].

Studies principally utilizing limited proteolysis have demonstrated that the E2 of the different complexes consists of several functional domains [8–14]. These include a compact, inner catalytic domain which, in addition to containing the active site, binds to other E2 catalytic domains to maintain the central core and also is responsible for binding of the E1 component of the complexes. The second type of domain is an extended, outer domain which contains the lysine group to which the lipoic acid cofactor is attached via a thioester linkage. The lipoate domains apparently interdigitate between the E1 and E3 components. Each E2 also contains a distinct region of polypeptide, thought to be present as a folded domain, which is responsible for binding of the E3 components [15]. The different domains are joined by linker regions of polypeptide, which vary in length and are characterized by being rich in proline, alanine and charged amino acid residues [16]. ^{1}H-n.m.r. spectroscopy has shown that these linker regions are extremely flexible, giving highly characteristic sharp resonances [17,18], which are diminished in genetically engineered complexes lacking some of the linker regions [19]. Furthermore, additional resonances are detected when a histidine residue is introduced, again by site-directed mutagenesis, into one of the linker regions [20].

The complexes vary in the number of lipoate domains which are contained within the E2 polypeptide. E2 from PDC of *Escherichia coli* is remarkable in that it contains three highly-conserved lipoate domains, located in tandem repeat at the *N*-terminus of the polypeptide [16]. Surprisingly, deletion of two of the three lipoate domains by site-specific mutagenesis still allows assembly of a functional complex with full catalytic activity [21]. Clearly the additional domains are not required for the catalytic functioning of the complex, which of course raises the interesting question of the function of the two additional lipoate domains.

Mammalian OGDC and BCOADC have a single lipoate domain and, although there is some evidence from isotope dilution studies that mammalian PDC also has a single lipoate residue [22], there is recent evidence that the E2 polypeptide has two lipoate residues [23]. This is indicated by cross-linking studies using phenylene-*O*-bismaleimide, in the presence of 2-oxo acid substrate, to form cross-links between lipoate residues. Whereas the E2 of OGDC is able to form only dimers, consistent with the presence of a single lipoate, E2 of PDC is apparently capable of cross-linking to form dimers, trimers and higher aggregates, an observation best explained by the presence of two or more lipoate residues. Furthermore the predicted protein sequence of a partial cDNA clone of rat liver PDC E2 [24] is consistent with the possible presence of a second lipoate domain (see below). A schematic representation of the domain structure of the different E2 components is given in Fig. 2.

The situation with mammalian PDC is further complicated by the presence of an additional component X which co-purifies with, and is tightly bound to, E2 [25,26]. Previously it was generally assumed that protein X was merely a proteolytic fragment of E2 but there is now convincing immunological and protein chemical data indicating that X is a distinct polypeptide. Antibodies raised against E2 fail to recognize X and *vice versa*, and limited proteolytic digestion yields a different pattern of peptides derived from E2 and X [25]. Recently

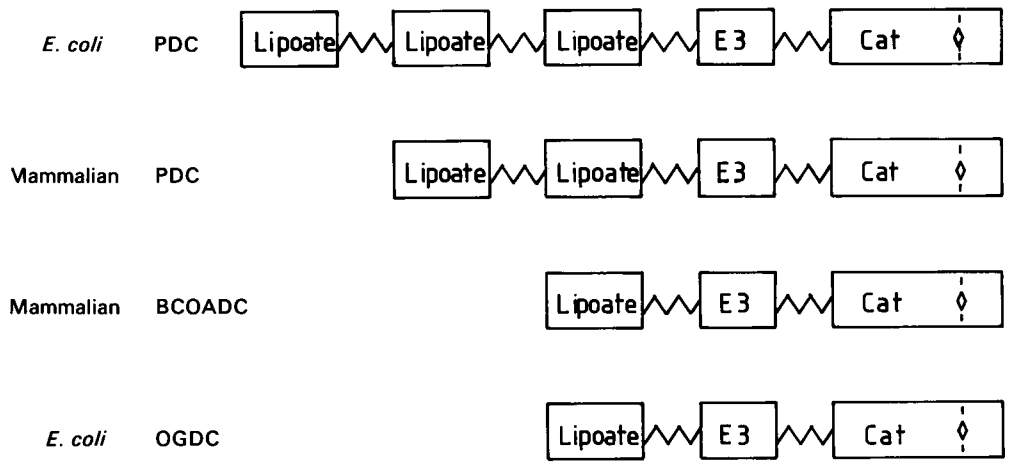

Fig. 2. Schematic representation of the domain structure of the E2 components

Lipoate indicates the lipoate-containing domain, E3 the E3-binding region and Cat the catalytic, core domain. The linker regions are indicated by the zigzags and the diamonds indicate the approximate position of the putative active sites.

Table 1. Primary structure surrounding lipoyl-lysine residues in proteins

Source	Enzyme	Sequence
Rat, bovine	PDC E2	V/I E T D *K A T V/I G F E V Q E E G
Bovine	OGDC E2	I E T D *K T S V Q V P S P A N G
Bovine, human	BCOADC E2	V Q S D *K A S V T I T S R Y D G
E. coli	PDC E2	V E G D *K A S M E V P A/S P F/Q A G
E. coli	OGDC E2	I E T D *K V V L E V P A S A D G
Chicken	Glycine-cleavage H-protein	L E S V *K A A S E L Y S P L T G

* Denotes the lysine residue to which the lipoate is attached.

it has been shown that protein X contains at least one lipoate moiety which can be reductively acetylated [27]. One possibility is that X is an isoenzymic form of E2 but the observation that E2 and X are present in approximately the same ratio in nine different rat tissues argues against X being a tissue-specific isoenzyme [23]. As the other complexes (apparently) do not possess a protein corresponding to X it seems unlikely that X is necessary for the catalytic functioning of mammalian PDC. Evidence has been presented that X is responsible for binding of the PDC kinase to the complex [28] and that the oxidative state of the lipoic acid residue in X serves a regulatory function by controlling the activity of the PDC kinase [29], but this has yet to be firmly established.

Surprisingly little is known concerning the enzymes responsible for attachment and removal of the lipoic acid cofactor. Whilst it seems likely that the ligase responsible for attachment of the lipoate is located within mitochondria there is no direct evidence in support of this. The sequence surrounding the lipoate attachment site on several bacterial and mammalian complexes has now been determined [16, 30–33], as has the lipoate-containing H-protein of the glycine cleavage system [34]. The lipoate-containing regions are highly conserved (Table 1), suggesting that the ligase recognizes some features of the primary structure. However this is a poorly understood area and it is not clear for example whether there is a single ligase responsible for insertion of the lipoates into the various substrate proteins. Is the lipoate attached prior to folding and assembly of the E2 polypeptide? Is the cofactor re-cycled during proteolytic degradation of the complexes?

In contrast to E2, the E1 and E3 are relatively simple enzymes! E3 is an FAD-containing flavoprotein and contains a disulphide bridge which undergoes reduction–oxidation during catalysis. The enzyme consists of two identical polypeptides of apparent molecular mass of approx. 55000. The E1 of bacterial and mammalian OGDC is also a homodimer (polypeptide size approx. 110000) but the E1 components of mammalian PDC and BCOADC are tetramers consisting of two copies of two non-identical subunits termed α and β. There is some evidence that the α-subunits catalyse reaction 1 and the β-subunits catalyse 2 (Fig. 1), but this is not yet rigorously established.

Molecular cloning of the bacterial and mammalian complexes

A major development in the study of the complexes was the work of John Guest and co-workers who cloned and sequenced the genes encoding the components of PDC and OGDC of *E. coli*, namely the *aceE* (E1, PDC) [35], *aceF* (E2, PDC) [16], *sucA* (E1, OGDC) [36], *sucB* (E2, OGDC) [30] and *lpd* (E3) genes [37]. Analysis of the predicted protein sequences of the E1 components of PDC and OGDC shows, perhaps surprisingly, very little similarity with each other, whereas the E2 components exhibit a high degree of similarity (for recent reviews see [7,38]). Furthermore, analysis of the amino acid sequences reveals similarity with several chloramphenicol acetyltransferases and has led to a proposed catalytic mechanism, involving a histidine residue in each E2 as a likely active site residue [39].

Very recently, several papers have been published reporting the molecular cloning and sequence analysis of cDNAs encoding all or part of the constituent polypeptides of the components of the eukaryotic 2-oxo acid dehydrogenase complexes.

To date, three groups have reported the cloning and sequencing of a full-length cDNA encoding the α-subunit of the E1 of human PDC. All three reported sequences are significantly different [40–42]! However, several important consistent features are found in each reported sequence: (1) the mature polypeptide is produced after cleavage of a 29-residue leader sequence necessary for mitochondrial targetting, (2) the phosphorylation sites lie in the *C*-terminal half of the polypeptide, and (3) there is no significant similarity between the α-subunits and the single subunit of the E1 components of the bacterial PDC and OGDC. A cDNA encoding the α-subunit of E1 of BCOADC from rat liver has also been cloned and sequenced [43] and analysis of the predicted protein sequence indicates a high degree of similarity with the α-subunit of PDC. Again, a leader sequence, this time of 40 amino acid residues, is present and again the phosphorylation sites lie in the *C*-terminal segment of the protein. Perhaps surprisingly there is as yet no sequence information available concerning the single subunit of E1 of the mammalian OGDC, and it will be interesting to see whether that polypeptide exhibits significant similarity to its prokaryotic counterpart.

Molecular cloning and sequence analysis of cDNA molecules encoding all or part of the E2 components of mammalian PDC and BCOADC has also been reported recently [24,33,44]. The rat liver PDC E2 protein shows a high degree of similarity both with its counterpart from *E. coli* and with the E2 component from human and bovine BCOADC. The reported sequence for E2 of PDC apparently encodes most of the polypeptide but is lacking the majority of the *N*-terminal lipoate domain and a small section at the *C*-terminal end of the polypeptide, whereas the BCOADC clone is lacking the sequence corresponding to the majority of the catalytic domain. However, the BCOADC E2 clone indicates that an extended leader sequence of 56 residues is present [44]. Molecular cloning of full-length cDNAs for both human [45] and yeast [46] E3 polypeptide has also been reported. These sequences indicate that E3 is a highly conserved protein, with about 41% overall identity in the protein sequence of yeast and *E. coli* and 44% identity between human and *E. coli*, with even higher conservation of sequence around the cysteine residues of the active site. Rat liver mitochondria contain two forms of E3 which can be distinguished immunologically [47] and it has been suggested that the second form is specifically involved in the glycine cleavage system, a second enzyme system which utilizes lipoic acid as an essential cofactor [34].

Biosynthesis and import of the eukaryotic complexes

All the subunits of the mitochondrial complexes are encoded by genes in the nucleus. The nascent polypeptides must therefore be imported into the mitochondrial matrix across two membranes, a process which is energy-dependent and which involves proteolytic cleavage of the polypeptides [48]. Furthermore, the appropriate cofactors must be inserted into the constituent polypeptides which must fold and aggregate into the mature correctly assembled complexes.

Current information indicates that all of the constituent polypeptides of the complexes (with the possible exception of component X of PDC) are cleaved near to their *N*-terminus during import, but the size of the cleaved fragment seems to vary considerably. As discussed above, comparison of the sequence predicted from cDNA sequences for the α [40–42] and β [41] subunits of PDC E1 and the E1 α-subunit of BCOADC [41] with *N*-terminal protein sequences of the mature polypeptides indicates that leader sequences of approx. 30 amino acids are removed during import. This is consistent with data from cultured cells which show that, in the presence of uncoupler to inhibit uptake and processing, precursors of the α and β subunits of PDC E1 accumulate which have an apparent molecular masses approx. 3 kDa greater than those of the mature polypeptides [49]. Similarly, precursors corresponding to the individual subunits of OGDC accumulate in the cytoplasmic fraction when cells are maintained in the presence of uncouplers [50]. The apparent molecular masses of the precursor forms of E2 from PDC and OGDC are approx. 8 kDa greater than those of the mature E2s [49,50], but unfortunately the cleavage point has not yet been confirmed as no sequence information on the *N*-terminus of the mature E2 polypeptides has yet been obtained. However, these large differences in apparent size between the precursor and mature forms of the E2 components are consistent with the presence of a large signal peptide of 56 residues on the E2 of BCOADC [44].

Phosphorylation of eukaryotic PDC and BCOADC

The activities of both mammalian PDC and BCOADC are subject to regulation by reversible phosphorylation [2,51]. In each case the effect of phosphorylation is to cause inactivation of the complex by a dramatic reduction of the $V_{max.}$ It appears that the phosphorylated form is catalytically inactive as no allosteric activators of the phosphorylated complexes have been found. In both complexes phosphorylation occurs exclusively on the α-subunit of E1. In PDC, three serine residues on the α-subunit undergo phosphorylation [52,53], whilst two sites on the α-subunit of BCOADC are subject to phosphorylation [54–56]. In each complex, phosphorylation of one site is primarily responsible for inactivation and the role of the additional sites has yet to be firmly established, although there is evidence that the additional sites on PDC may inhibit dephosphorylation of the inactivating site [57].

As yet there is no convincing evidence for phosphorylation of PDC from yeast. However the E1 component of yeast PDC can be phosphorylated *in vitro* by bovine kidney PDC kinase and the site of phosphorylation is very similar in primary structure to the major inactivating site in the mammalian complex [58], indicating that the yeast complex at least has the potential to be regulated by reversible phosphorylation.

Properties of the relevant protein kinases and phosphatases

The kinases and phosphatases acting on PDC and BCOADC are specific for each complex and appear distinct from any of the cytoplasmic kinases or phosphatases, with the exception of a recently discovered mitochondrial PDC phosphatase termed phosphatase SP [59]. However, as no primary structure information is available for any of the regulatory enzymes, it may yet turn out that these mitochondrial enzymes are related to some of the well-characterized cytoplasmic protein kinases and phosphatases. The kinases are tightly bound to, and copurify with, their respective complexes, whereas the phosphatases are more loosely associated and are purified as distinct, soluble enzymes.

PDC kinase has been purified to homogeneity and its structure elucidated [60]. It consists of two non-identical subunits termed α and β, which have molecular masses of 48000 and 45000 respectively, and the kinase is apparently a dimer. Limited proteolytic studies have indicated that the α-subunit contains the kinase active site and it has been postulated that the β-subunit may serve a regulatory role [60] but there is no evidence in direct support of this. PDC kinase is specific for PDC and has little activity against other protein substrates, including BCOADC. The activity of the kinase is stimulated by acetyl-CoA and NADH, products of the complex, and this stimulation is antagonized by the substrates CoA and NAD^+. It is also inhibited by pyruvate and by thiamin pyrophosphate, which presumably binds directly to the active site of the E1 substrate (for a recent review, see [51]).

The homogeneous preparations of PDC kinase in [60] were obtained from an E2–kinase subfraction of the complex. It has been reported more recently that a significant proportion of the kinase remains associated

with the E1 component and not with E2 during resolution of the complex [61]. Whatever the explanation for these differences, it seems that the kinases associated with E1 or E2 are functionally and structurally indistinguishable (K. G. Cook, P. Dent, S. P. M. Fussey & S. J. Yeaman, unpublished work).

By comparison, little is known concerning the BCOADC kinase. It remains largely associated with E2 during resolution of the complex [62], but methods have not yet been devised to subsequently dissociate it from E2 and the kinase polypeptide(s) have not been identified or purified. The major regulatory mechanism for the BCOADC kinase is inhibition by the 2-oxo acid substrates of the complex [4,63–65]. Perhaps surprisingly, the 2-oxo acids inhibit phosphorylation of site 2 more than that of site 1 (the major inactivating site) [56], but the significance of this has yet to be determined. The kinetics of inhibition by the 2-oxo acids are complex, being mixed or non-competitive [65]. All five known 2-oxo acid substrates are inhibitory, with 4-methyl-2-oxopentanoate being most potent and 3-methyl-2-oxobutyrate being significantly less potent than the others [4]. It is noteworthy that the activity of the kinase is apparently insensitive to the NAD^+/NADH and CoA/acetyl-CoA ratios [65].

PDC phosphatase has been purified to homogeneity and its structure characterized [66,67]. It has a molecular mass of approx. 150000 and consists of two subunit types, the catalytic activity residing in a subunit of 50000 [66]. The larger subunit of 97000 contains a molecule of FAD, but the function of this cofactor remains unknown. The phosphatase is highly specific for PDC, with minimal activity against phosphorylated BCOADC [68], and the purified phosphatase has an absolute requirement for Mg^{2+} ions and is stimulated, via reduction in the K_m for its E1 substrate, by micromolar concentrations of Ca^{2+} ions, the Ca^{2+} ions apparently acting by promoting binding of the phosphatase to the E2 core of the complex (for recent reviews, see [51,69]).

BCOADC phosphatase has also been obtained in a highly purified form [68], with the native enzyme having a molecular mass of approx. 460000. The subunit composition has not been determined, but a catalytic subunit of M_r 33000 has been purified to homogeneity following disruption of the native enzyme by 6 M-urea [70]. The isolated catalytic subunit possesses apparently identical catalytic and regulatory properties as the M_r 460000 native enzyme, raising the question of the function of the other, as yet uncharacterized, subunits of the enzyme. Both the native enzyme and the isolated catalytic subunit are inhibited, in a non-competitive manner, by a potent inhibitor protein of M_r 36000, which has a subnanomolar inhibitor constant [71]. This protein, which is heat- and acid-stable, is distinct from the cytosolic inhibitors 1 and 2 of protein phosphatase 1 [72] and its effects on BCOADC phosphatase are reversed by Mg^{2+} ions [71]. BCOADC phosphatase has significant activity against phosphorylated PDC (10% of that against phosphorylated BCOADC) but, as it is present in mitochondria in much lower amounts than PDC phosphatase, it is unlikely to act as a physiologically important PDC phosphatase.

A third mitochondrial protein phosphatase, termed phosphatase SP (spermine-activated) has recently been identified and characterized [59]. It has been purified approx. 15000-fold and consists of two subunit types with apparent M_r values of 60000 and 34000. It can be dissociated in the presence of urea and NaCl and it appears that the M_r 34000 subunit possesses the catalytic activity. This activity is inhibited in a specific manner by polyclonal antibodies raised against the cytoplasmic protein phosphatase 2A [73], suggesting that it is related to phosphatase 2A, but molecular cloning and sequencing of the catalytic subunit of phosphatase SP will be required to clarify this possibility. It has been estimated that phosphatase SP accounts for approx. 25% of the phosphatase activity against phosphorylated PDC in mitochondrial extracts, and clearly this phosphatase may be of major physiological significance.

Acute control of the mammalian complexes

The activities of both OGDC and PDC are subject to control by changes in the intramitochondrial concentrations of free Ca^{2+} ions [74]. The effect of Ca^{2+} ions on OGDC is apparently via direct binding of the Ca^{2+} ions to the complex, causing allosteric activation by reducing the K_m for the substrate 2-oxoglutarate [75,76]. This effect is antagonized by ATP, which increases the K_m for 2-oxoglutarate. This mechanism is thought to underly the activation of OGDC by Ca^{2+}-mobilizing hormones such as adrenaline in heart and vasopressin and the α-adrenergic action in liver [74]. Such Ca^{2+}-mobilizing hormones also activate PDC via increases in the intramitochondrial levels of free Ca^{2+} ions. However, the activation of PDC is not via direct allosteric activation, but is via stimulation of PDC phosphatase [74]. Evidence in support of this has come from work principally with perfused rat hearts and with isolated heart mitochondria [77–79]. In particular, the presence of Ruthenium Red, a specific inhibitor of Ca^{2+} ion uptake into mitochondria, blocks the activation of PDC in hearts perfused with positive inotropic agents such as adrenaline or isoprenaline [78]. An intriguing observation is that the effect of Ca^{2+} ions on the activity of PDC phosphatase within mitochondria is very different than on the isolated enzyme in dilute solution [80,81] in that, within mitochondria, it seems that Ca^{2+} ions are without effect on the activity of the phosphatase at saturating concentrations of Mg^{2+} ions and that the major effect of Ca^{2+} ions is to lower the K_a for Mg^{2+} ions.

The activity of BCOADC is apparently insensitive to influence by Ca^{2+} ions, either directly or via effects on BCOADC kinase and/or phosphatase. Indeed there is a paucity of convincing data on acute hormonal effects on that complex. However there is a report that administration of adrenaline or glucagon to rats fed a low-protein diet causes a dramatic increase in the amount of active BCOADC in liver [82]. This effect can be mimicked by cyclic AMP, but the mechanism remains unknown. It may be relevant that no acute effects of hormones were observed on the extent of phosphorylation of BCOADC in hepatocytes from low-protein fed rats [83], perhaps suggesting that the hormones act via an indirect mechanism in whole animals. Very recently, studies with perfused rat hearts have indicated that glucagon or the β-adrenergic action of adrenaline causes a very rapid inactivation of BCOADC, but α-adrenergic agents cause a slower, prolonged activation of the enzyme complex [84].

A major mechanism for acute control of the activity of BCOADC is via inhibition of the BCOADC kinase by the substrate 2-oxo acids. Consistent with the observed inhibition *in vitro* of the kinase intrinsic to the purified

complex [4,63–65], it has been shown, using perfused hearts [85] and hepatocytes isolated from rats fed a low-protein diet [86,87], that flux through the complex is increased by the presence of 2-oxo acid substrates in the incubation medium. This results from activation of the complex, observed as an increase in the activity of the complex in extracts prepared from perfused hearts or hepatocytes [65,86]. Using specific antibodies to immunoprecipitate E1 from extracts prepared from cells pre-incubated with [^{32}P]P_i, decreased phosphorylation of the α-subunit of E1 at both phosphorylation sites in response to 2-oxo acids has been demonstrated directly in hepatocytes [83]. Similar experiments have also been carried out with isolated adipocytes [88].

The classical acute hormonal effect on the activity of the complexes is the dephosphorylation and activation of PDC in adipose tissue by insulin (reviewed in [51]), mediated via stimulation of PDC phosphatase [89] by an unknown mechanism which does not involve increases in the intramitochondrial concentrations of free Ca^{2+} ions [90]. The effects of insulin on the phosphatase persist during homogenization of the tissue, preparation of mitochondria and subsequent permeabilization of these mitochondria with toluene [91]. However these effects are lost in extracts prepared from these mitochondria [80,90]. The effect of insulin is to lower the K_a for Mg^{2+} ions [80,91], an effect mimicked on purified phosphatase by the polyamine spermine [80,92]. This has led to the suggestion that spermine or a spermine-like compound may mediate insulin's effect on the phosphorylation state of PDC [92], but as the insulin effect persists in mitochondria, which are likely to be permeable to spermine, and exogenous spermine has no effect on the activity of PDC phosphatase in these permeable mitochondria, it has been suggested that a high-M_r molecule, perhaps with some spermine-like properties, may mediate the intramitochondrial actions of insulin [91].

Long-term physiological regulation of PDC and BCOADC

The activities of mammalian PDC and BCOADC are under long-term control and vary in response to diabetes and diet. During long-term starvation (48 h) or in alloxan-induced diabetes, the activity state of PDC in heart, liver and kidney drops significantly [93,94]. This is primarily due to increased activity of PDC kinase, to which several mechanisms contribute. Increased lipid oxidation increases the ratios of acetyl-CoA/CoA and NADH/NAD^+, causing acute activation of the kinase [95], but there are also mechanisms for a stable increase in kinase activity [96–99]. It appears that there is an increase of 2–3-fold in the amount of kinase activity associated with the complex, but also a 2-fold increase in the amount of a kinase activator protein which can be resolved from the complex by gel filtration chromatography [98]. However, the activator protein has not yet been purified and fully characterized, and it cannot be firmly excluded that the kinase activator is in fact additional kinase molecules which dissociate from, or are unable to bind to, the complex. Such a conclusion must await purification of the activator and investigation of whether it possesses PDC kinase (or PDC kinase kinase!) activity.

The primary site of oxidation of branched-chain amino and 2-oxo acids is the liver. Other tissues such as heart and skeletal muscle contain significant levels of BCOADC, but the enzyme is primarily in the phosphorylated, inactive form [100–103].

In the liver of rats fed a standard chow diet with a protein content of approx. 20% or greater, the complex is essentially all in the active form, with an activity state of 90% or higher [101,103,104]. Feeding a diet with a restricted protein content leads to a reduction in the activity state [103–105]. The decrease occurs within 2–3 days and the final value obtained is related to the protein content of the diet [104]. Such a reduction in the activity of BCOADC in liver will allow conservation of the essential branched-chain amino acids during times of negative nitrogen balance. An explanation for the decreased activity state during times of restricted protein intake has been provided by the finding of a 4-fold increase in kinase activity under those conditions [106]. Clearly the mechanism responsible for these changes in kinase levels is an important outstanding question. There is also evidence that total activity of BCOADC is decreased in the livers of rats fed a diet low in protein [100,103,104] but this is contradicted by immunoassay studies utilizing antibodies raised against one or more of the components of the complex [105,107].

It now seems clear, from freeze-clamping studies, that during starvation the high activity state of BCOADC in liver is maintained, to allow utilization of branched-chain amino and 2-oxo acids as glucogenic and ketogenic precursors [103,105]. Furthermore, starvation of rats previously fed a low-protein diet leads to re-activation of the complex in liver, although the complex in heart is apparently insensitive to starvation [103]. Earlier data indicating that starvation causes a dramatic decrease in the activity state in liver [107] can be at least partly explained by the findings that, despite precautionary measures, the activity state can alter dramatically during preparation of mitochondria from starved animals [108]. Maintenance of the high activity state of the complex in liver upon starvation of rats fed previously a high-protein diet is consistent with the finding that levels of kinase activity are unaffected by starvation [106].

Role of complexes in primary biliary cirrhosis

Primary biliary cirrhosis is a cholestatic liver disease characterized by inflammatory obliteration of intrahepatic bile ducts, leading to liver cell damage and cirrhosis [109]. It is apparently an autoimmune process, characterized particularly by the presence of anti-mitochondrial antibodies in the sera of patients [110,111]. A variety of mitochondrial autoantigens have been reported [112,113] and their identities suggested but, until recently, no autoantigen had been conclusively identified. Two mitochondrial antigens of apparent M_r 70000 and 45000 on SDS/polyacrylamide-gel electrophoresis have been characterized [114] and using antisera from primary biliary cirrhosis patients to screen a rat liver cDNA library, the cDNA coding for the M_r 70000 antigen has been cloned and the resultant cDNA sequenced, although surprisingly this did not lead to identification of the autoantigen [24].

Detailed inspection of the protein sequence predicted from the cDNA allows identification of the autoantigen as the E2 component of PDC [115]. In particular, the predicted protein sequence contains a region corresponding to the site of attachment of the lipoic acid cofactor [31]. The identification of the autoantigen has been substantiated using immunoblotting of patients'

sera against purified bovine E2, where it was found that approx. 95% of primary biliary cirrhosis patients possess autoantibodies against E2. Interestingly, all sera from these patients also cross-react with protein X present in the preparations of E2 which were used [115], whilst sera from some patients recognize the E2 components of OGDC and BCOADC [116]. Whilst it is still unclear what role, if any, the presence of circulating antibodies plays in the pathogenesis of the disease, the availability of purified autoantigens may provide an insight in the mechanisms involved. Furthermore, the involvement of the 2-oxo acid dehydrogenase complexes in this autoimmune disease reveals yet another facet of these fascinating enzyme systems.

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