Mitochondrial 2-Oxoacid Dehydrogenase Complexes of Animal Tissues

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# Mitochondrial 2-oxoacid dehydrogenase complexes of animal tissues

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The pyruvate dehydrogenase and branched-chain 2-oxoacid dehydrogenase complexes of animal mitochondria are inactivated by phosphorylation of serine residues, and reactivated by dephosphorylation. In addition, phosphorylated branched-chain complex is reactivated, apparently without dephosphorylation, by a protein or protein-associated factor present in liver and kidney mitochondria but not in heart or skeletal muscle mitochondria. Interconversion of the branched-chain complex may adjust the degradation of branched-chain amino acids in different tissues in response to supply. Phosphorylation is inhibited by branched-chain ketoacids, ADP and TPP.

The pyruvate dehydrogenase complex is almost totally inactivated (99%) by starvation or diabetes, the kinase reactions being accelerated by products of fatty acid oxidation and by a protein or protein-associated factor induced by starvation or diabetes. There are three sites of phosphorylation, but only sites 1 and 2 are inactivating. Site 1 phosphorylation accounts for 98% of inactivation except during dephosphorylation when its contribution falls to 93%. Sites 2 and 3 are only fully phosphorylated when the complex is fully inactivated (starvation, diabetes). Phosphorylation of sites 2 and 3 inhibits reactivation by phosphatase. The phosphatase reaction is activated by Ca^{2+} (which may mediate effects of muscle work) and possibly by uncharacterized factors mediating insulin action in adipocytes.

Introduction

Mitochondrial 2-oxoacid dehydrogenase complexes catalyse reactions of the general type

$$RCOCOOH + NAD^+ + CoASH \xrightarrow{TPP, Mg^{2+}} RCOSCoA + NADH + H^+ + CO_2; \qquad (1)$$
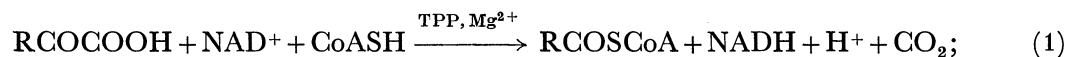
the equilibrium constant is approximately 10^6–10^7. *In vivo* the reactions are non-reversible and animal tissues lack other means of performing reversal. Regulation by reversible phosphorylation is confined currently to the pyruvate and branched-chain 2-oxoacid dehydrogenase complexes. There are no obvious reasons at present for suspecting that the 2-oxoglutarate dehydrogenase complex is regulated in this way.

Reversible phosphorylation of the pyruvate dehydrogenase complex was discovered by Linn *et al.* (1969*b*). Its discovery followed the observation of ATP-dependent inactivation in the course of attempts to stabilize the complex during purification (Reed 1981). The branched-chain complex (this abbreviation will be used throughout) was discovered comparatively recently by Danner *et al.* (1978), Parker & Randle (1978*a*) and Pettit *et al.* (1978). Interconvertible active and inactive forms of the complex in rat heart mitochondria were discovered by Parker & Randle (1978*b*). The discovery was occasioned by the observation that the activity of the complex in freshly prepared mitochondria was too low to account for rates of leucine oxidation in perfused heart. It had been assumed until then that muscle branched-chain complex was unstable and inactivated by extraction. Fatania *et al.* (1981) were the first to copurify

the complex and its kinase to apparent homogeneity and to show that inactivation by $Mg[\gamma\text{-}^{32}P]ATP$ is correlated with the incorporation of ^{32}P into the complex.

In vivo the pyruvate dehydrogenase complex catalyses the first reaction in glucose degradation for which no means of reversal is known in animals. The acetyl-CoA formed may be oxidized in support of ATP synthesis (pyruvate is the only source in some brain cells) or used for fatty acid synthesis in hepatocytes and adipocytes. Flux through the pyruvate dehydrogenase complex reaction is increased in muscles by mechanical work and in adipocytes and hepatocytes when insulin stimulates fatty acid biosynthesis. Flux is decreased in tissues generally by starvation and pancreatic diabetes; in muscles, liver, kidney and adipocytes by oxidation of fatty acids; and in muscles and some brain cells by oxidation of ketone bodies (some evidence is reviewed by Randle *et al.* 1978). The mechanisms that may be responsible, through reversible phosphorylation, for these changes in flux will be reviewed.

The branched-chain complex catalyses the first reaction in branched-chain amino acid degradation for which no means of reversal in animals is known. Branched-chain amino acids are essential amino acids in man and rat. In adult animals in nitrogen equilibrium the dietary requirement is presumably determined by the rate of oxidation of the corresponding 2-oxoacids by the branched-chain complex because transamination is not rate-limiting *in vivo* (for review see Krebs & Lund 1977). The disposal of excess branched-chain amino acids, whether derived from the diet or from breakdown of tissue protein, is a further important function of the branched-chain complex. This function is lost in maple-syrup urine disease in which a genetic variant of the branched-chain complex shows grossly reduced activity; if dietary intake of branched-chain amino acids is not curtailed, severe brain damage occurs and few affected infants survive beyond the second year of life. Evidence for toxicity of excessive intake of branched-chain amino acids in normal animals is reviewed by Harper *et al.* (1970).

Complete degradation of branched-chain amino acids leads to the formation of HMGCoA (from leucine) succinyl-CoA (from valine) and acetyl-CoA and succinyl-CoA (from isoleucine). Leucine and isoleucine are ketogenic and valine and isoleucine are glucogenic; this is of potential usefulness in starvation. It is not clear whether conversion to glucose and ketone bodies requires the total hepatic degradation of branched-chain ketoacids. It is known that transaminase activity is low in liver and gut, thus allowing branched-chain amino acids to pass freely into the peripheral circulation (see Krebs & Lund 1977). It is also known that transaminase activity exceeds branched-chain complex activity in muscles and that branched-chain ketoacids are released from muscles into the blood and taken up by the liver (Livesey & Lund 1980). The mechanisms that may be responsible, through reversible phosphorylation of the branched-chain complex, for adjusting rates of degradation in response to input of branched-chain amino acids and for the disposition between hepatic and extrahepatic tissues will be discussed.

Chemistry and 2-oxoacid specificity of the complexes

Both complexes contain multiple copies of three enzymes: E_1, a 2-oxoacid decarboxylase (or dehydrogenase) forming hydroxyalkyl TPP-E_1 and CO_2; E_2, an acyltransferase forming acyl-CoA; and E_3, lipoyl dehydrogenase (common to both complexes) forming NADH; and lipoyl residues attached covalently to E_2 transfer acyl groups and H between component enzymes of the complex (for reviews see Reed (1981) and Randle *et al.* (1983)). In pyruvate dehydrogenase complexes of animal tissues the E_1 component has two dissimilar subunits ($M_r(\alpha) = 41\,000$;

$M_r(\beta) = 36\,000$) and is a tetramer ($\alpha_2\beta_2$). The E_2 component contains 60 copies of a subunit of M_r 52 000 arranged as a pentagonal dodecahedron. The E_3 component is a dimer of a subunit of M_r 55 000. As purified, the E_2 core carries either 30 or 60 E_1 tetramers and 12E_3 dimers. M_r values are by sodium dodecyl sulphate polyacrylamide gel electrophoresis except for E_2, which because of an extended configuration gives an anomalous M_r (76 000); the figure of 52 000 is based on sedimentation equilibrium (Barrera *et al.* 1972; Sugden & Randle 1978). In branched-chain complexes the E_1 component has two dissimilar subunits ($M_r(\alpha) = 46\,000$; $M_r(\beta) = 31\,000$–35 000); the E_2 component is a cube composed of multiple copies of a subunit of M_r 52 000; M_r values are by sodium dodecyl sulphate polyacrylamide gel electrophoresis (Pettit *et al.* 1978; Lau *et al.* 1982; Odyssey 1980*b*, 1982).

The 2-oxoacid substrates for the pyruvate dehydrogenase holocomplex reaction are pyruvate, hydroxypyruvate and 2-oxobutyrate. The 2-oxoacid substrates for the branched-chain complex are the 2-oxoacids corresponding to leucine, isoleucine, valine and methionine (referred to as ketoleucine, L (or D) ketoisoleucine, ketovaline and ketomethionine), 2-oxobutyrate and pyruvate. Pyruvate is a poor substrate; the K_m is 20 times the K_m for the pyruvate dehydrogenase complex and the V_{max} is lower. Apparent K_m values for the principal physiological substrates are 50 μM (pyruvate dehydrogenase complex) and 10–50 μM branched chain ketoacids (branched chain complex) (Danner *et al.* 1978; Parker & Randle 1978*a*, *b*; Pettit *et al.* 1978; Randle *et al.* 1981).

Both complexes are inhibited by their principal end-products, acyl-CoA (competitive with CoA) and NADH (competitive with NAD+) (Garland & Randle 1964; Randle *et al.* 1966; Parker & Randle 1978*a*; Pettit *et al.* 1978).

REVERSIBLE PHOSPHORYLATION IN THE PYRUVATE DEHYDROGENASE COMPLEX

Phosphorylation is confined to serine residues in the α chain of the E_1 component and appears to be half-site in ox and pig complexes, i.e. it is equivalent to only one α chain of the E_1 component (Reed 1981; Sugden & Randle 1978). Phosphorylation results in more than 99 % inactivation and reactivation can only be induced by dephosphorylation. Phosphorylation may be followed in the purified complex (which contains the kinase) or in mitochondria or in tissues, by assaying holocomplex activity; and by incorporation of ^{32}P into the complex from [γ-^{32}P]ATP (the latter may be generated *in situ* from $^{32}P_1$ in mitochondria or isolated tissues). Close parallelism exists between the behaviour of the purified complex, and the complex in mitochondria or isolated tissues.

The pyruvate dehydrogenase kinase reactions

Chemistry

Pyruvate dehydrogenase kinase has been separated from the complex and purified to apparent homogeneity. It is composed of two dissimilar subunits ($M_r = 45\,000$ and 48 000) attached to the E_2 core and is an SH enzyme (Reed & Pettit 1981; Pettit *et al.* 1982). It is specific for ATP or its thiophosphoryl analogue (Reed & Pettit 1981; Tonks *et al.* 1982). Three serine residues are phosphorylated; these are recovered in two tryptic phosphopeptides, the structure of which (scheme 1*a*, *b*) are shown for pig (Sugden *et al.* 1979). Ox complex has asparagine in place of aspartate in (*a*) (Yeaman *et al.* 1978). Cleavage of the Asp-Pro bond in

Tyr-His-Gly-His-Ser-Met-Ser-Asp-Pro-Gly-Val-Ser-Tyr-Arg (a)

site 1   site 2

Tyr-Gly-Met-Gly-Thr-Ser-Val-Glu-Arg (b)

site 3

Scheme 1

(a) allows site occupancy to be determined (Sale & Randle 1981b, 1982a). Phosphorylation sites 1 or 2 are inactivating; site 3 is not (Reed & Pettit 1981; Sale & Randle 1982a; Tonks *et al.* 1982). During phosphorylation or in the steady state *in vivo*, more than 98 % of inactivation is due to phosphorylation of site 1 and less than 2 % to site 2 (Sale & Randle 1981a, b, 1982a, b). The major function of sites 2 and 3 is therefore not inactivation.

Regulation

The kinase reactions are inhibited by ADP (competitive with ATP) and by pyruvate (uncompetitive with ATP) and by salts of dichloroacetic acid and some other halogenated carboxylic acids (Linn *et al.* 1969a, b; Cooper *et al.* 1974; Whitehouse *et al.* 1974). The kinase reactions are accelerated by increasing concentration ratios of $NADH/NAD^+$ and acetyl-CoA/CoA (Pettit *et al.* 1975; Cooper *et al.* 1975; Kerbey *et al.* 1979). Evidence for operation of these regulatory mechanisms in mitochondria is given by Hansford (1976), Kerbey *et al.* (1977) and Sale & Randle (1980). Regulation of the kinase reaction by kinase/activator protein is discussed in a later section on starvation and diabetes.

The pyruvate dehydrogenase phosphate phosphatase reactions

Chemistry

Pyruvate dehydrogenase phosphatase is a dimer of two subunits ($M_r = 50\,000$ and $98\,000$) (Reed & Pettit 1981) and is readily separated from the complex ($M_r \approx 10^7$) by fractional precipitation or differential centrifugation. Relative rates of dephosphorylation of the three sites of phosphorylation are site 2 > site 1 ⩾ site 3 (purified complex or in mitochondria) (Teague *et al.* 1979; Kerbey *et al.* 1981); Sale & Randle 1982a). Thiophosphoryl complexes are resistant to phosphatase action (Tonks *et al.* 1982).

Regulation

The phosphatase requires Mg^{2+} ($K_{0.5}$ approximately 1 mM) and in the presence of Mg^{2+} is activated by Ca^{2+} ($K_{0.5}$ approximately 1 μM) (Denton *et al.* 1972; Randle *et al.* 1974). Both divalent metal ions are required for phosphatase activity in mitochondria. Extramitochondrial Ca^{2+} activates the phosphatase reaction (presumably by raising intramitochondrial Ca^{2+} concentration) and $K_{0.5}$ is approximately 0.5 μM (Denton *et al.* 1980; Sale & Randle 1982a).

In a substantial number of studies in my laboratory it has been found that phosphorylation of sites 2 and 3 in the complex inhibits the reactivation and dephosphorylation of site 1 by phosphatase. The initial rate of reactivation of partly phosphorylated complex (80–90 % site 1) was approximately five times that of fully phosphorylated complex (Sugden *et al.* 1978; Kerbey & Randle 1979; Sugden & Simister 1980; Kerbey *et al.* 1981). Thiophosphorylation of sites 2 and 3 also inhibited dephosphorylation of site 1 (Tonks *et al.* 1982). During dephosphorylation the contribution of site 2 to inactivation increases to 7 %, supporting a role for this site in inhibiting reactivation (Sale & Randle 1982a). However, Teague *et al.* (1979) were unable to

show any effect of phosphorylation of sites 2 and 3 on the rate of reactivation of the complex or the dephosphorylation of site 1. The reason for this discrepancy is not clear. The only obvious difference, experimentally, is that Teague *et al.* used phosphatase purified to apparent homogeneity. Our own preparations of phosphatase were not so pure. Whether some other factor, lost in the final stages of purification of the phosphatase, is involved in this inhibitory effect of site 2 and 3 phosphorylations on reactivation by phosphatase remains to be investigated. There is evidence that phosphorylation of sites 2 and 3 in mitochondria may inhibit reactivation (Sale & Randle 1980).

Occupancy of phosphorylation sites in vivo

In vivo, kinase and phosphatase reactions operate simultaneously. Thus, inhibitors of the kinase reactions induce dephosphorylation and reactivation (Whitehouse *et al.* 1974), and in mitochondria phosphate in pyruvate dehydrogenase phosphate turns over in the steady state (Sale & Randle 1982a). Occupancy of phosphorylation sites is therefore the difference of the kinase and phosphatase reactions. It has been measured in mitochondria with $^{32}P_i$ (Sale & Randle 1980, 1982a) and in heart muscle *in vivo* by back-titration of unoccupied sites with $[\gamma\text{-}^{32}P]ATP$ or by perfusion with $^{32}P_i$ (Sale & Randle 1981a, 1982b). The pattern of occupancy is the same in both mitochondria and heart. Occupancy of site 1 was linearly correlated with the proportion of complex in the inactive form. Occupancy of the other two sites (2 and 3) lagged behind that of site 1 up to approximately 70% of inactive complex; over this range relative occupancy of the three sites was approximately constant (1:0.6:0.4 for sites 1:2:3). Between 70 and 100% of inactive complex, occupancy of sites 2 and 3 increased relative to site 1 and approximated to equivalence when all complex was inactive. In fed normal rats the occupancy of sites 2 and 3 was at its minimum; in starved and diabetic rats the occupancy of sites 2 and 3 was at its maximum. It is suggested that this is a hysteresis mechanism that serves to restrain reactivation of phosphorylated complex in starvation. The relative initial rates of reactivation of phosphorylated complexes purified from hearts of fed or starved rats was approximately 3:1 (fed/starved) (Sale & Randle 1982b).

Physiopathology of reversible phosphorylation

The mechanisms that may change the relative activities of kinase and phosphatase and hence the proportion of active complex in tissues is reviewed briefly below.

Muscle contraction

In skeletal muscle (Hennig *et al.* 1975) and in heart, work increases the proportion of active complex. The major factor is assumed to be increased cytosolic Ca^{2+} concentration; over the concentration range 10^{-8} to 10^{-6} M extramitochondrial Ca^{2+} activates the phosphatase in mitochondria (Denton *et al.* 1980; Sale & Randle 1982a). Inhibition of the kinase by diminished mitochondrial concentration ratios of ATP/ADP, acetyl-CoA/CoA and NADH/NAD$^+$ may be a contributory factor.

Lipid fuels

Oxidation of fatty acids or ketone bodies, or both, decreases the proportion of active complex in muscles (Wieland *et al.* 1971b; Hagg *et al.* 1976; Kerbey *et al.* 1976). This is attributed to activation of the kinase reaction by increased mitochondrial concentration ratios of acetyl-CoA/CoA and NADH/NAD$^+$ (Garland & Randle 1964; Randle *et al.* 1966; Pearce *et al.* 1979).

Insulin action

Insulin increases the proportion of active complex in adipocytes *in vitro* (Jungas 1970; Coore *et al.* 1971) but not in heart or skeletal muscle *in vitro* (Caterson *et al.* 1982). This action in adipocytes appears to result from activation of the phosphatase (Hughes & Denton 1976), perhaps as the result of activation by a peptide mediator of insulin action (Larner *et al.* 1982).

Starvation (48 h) *and alloxan diabetes*

Starvation and alloxan diabetes decrease the proportion of active complex in tissues generally. The effects of starvation are reversed by feeding, and those of diabetes by insulin *in vivo*, but not by insulin *in vitro* (Wieland *et al.* 1971 a; Caterson *et al.* 1982). Fatty acid oxidation is essential for this effect of starvation and alloxan diabetes in heart muscle and kidney, because the effect can be reversed by inhibiting fatty acid oxidation with 2-tetradecylglycidic acid (Caterson *et al.* 1982). The role of fatty acid oxidation in liver, kidney and adipocytes has yet to be established.

Studies by Kerbey *et al.* (1976, 1977) showed that the effect of starvation and diabetes to decrease the proportion of active complex persists into mitochondria prepared from the heart. This difference is most obvious when mitochondria are incubated in the presence of inhibitors of the kinase, and is not explicable in terms of known effectors of the kinase or of the phosphatase. Hutson & Randle (1978) showed that the kinase reaction is faster than in controls in extracts prepared from heart mitochondria of starved or alloxan-diabetic rats. Similar observations in respect of starvation were made employing extracts of mammary-gland mitochondria by Baxter & Coore (1978). More recently a protein factor termed kinase/activator, which accelerates the pyruvate dehydrogenase kinase reaction, and which may be induced by starvation of the rat, has been separated from the complex in extracts of mitochondria (Kerbey & Randle 1981, 1982). The factor has not been fully characterized but it appears to be a protein (or protein-associated factor) of M_r 100 000 or greater.

Reversible phosphorylation in the branched-chain complex

This topic has been reviewed recently in more detail (Randle *et al.* 1983). Phosphorylation is confined in ox and rat complexes to serine residue(s) in the α chain of the E_1 component (Lau *et al.* 1982; Odyssey 1982). The stoichiometry of the phosphorylation is not known. Preliminary studies based on tryptic digestion suggest more than one site of phosphorylation (Randle *et al.* 1983). Phosphorylation results in more than 98 % inactivation; reactivation may be induced by dephosphorylation or without detectable dephosphorylation by a mitochondrial factor called, provisionally, activator protein. Phosphorylation may be followed in purified complex (with which the kinase may be copurified) or in mitochondria, by assaying holo-complex activity or incorporation of ^{32}P from $[\gamma\text{-}^{32}P]ATP$ into the complex. The concentration of branched-chain complex in mitochondria is much lower than that of the pyruvate dehydrogenase complex. In the rat, the activity of branched-chain complex (milliunits per milligram of protein) is approximately 10 (liver), 4–6 (kidney) and 3 (heart or skeletal muscle) (Randle *et al.* 1983), whereas that of pyruvate dehydrogenase complex is approximately 50 (liver), 50 (kidney) and 75–120 (heart or skeletal muscle). To demonstrate the incorporation of ^{32}P into the complex in mitochondria it is necessary to inhibit incorporation into the pyruvate

dehydrogenase complex (with pyruvate or dichloroacetate) and to separate the α chains of the two complexes by sodium dodecyl sulphate polyacrylamide gel electrophoresis in Tris–glycine buffers (Randle *et al.* 1981).

The branched-chain dehydrogenase kinase and phosphatase reactions

The earliest procedures for purification of the branched-chain complex to apparent homogeneity resulted in loss of kinase activity. This led to the conclusion that the branched-chain complex is not regulated by reversible phosphorylation (Pettit *et al.* 1978; Danner *et al.* 1979). Quite independently, and at the same time, studies with heart mitochondria showed the existence of interconvertible active and inactive forms, and of inactivation by MgATP (Parker & Randle 1978*b*). Experiments with mitochondria and with mitochondrial and tissue extracts from rat heart, skeletal muscle, liver and kidney established that the branched-chain complex is inactivated by ATP, and that inactivation is associated with the incorporation of ^{32}P from $[\gamma\text{-}^{32}$P]ATP into a protein of M_r 46000. This protein was identified tentatively as the α chain of the decarboxylase component (Odyssey & Goldberg 1979; Parker & Randle 1980; Odyssey 1980*a*, *b*; Lau *et al.* 1981; Hughes & Halestrap 1981). Fatania *et al.* (1981) first developed a method of copurifying the ox kidney complex and its intrinsic kinase to near-homogeneity and showed a strict correlation between inactivation and ^{32}P incorporation. They confirmed also that procedures used by Pettit *et al.* (1978) and Danner *et al.* (1979) led to loss or inactivation of the kinase. Copurification of rabbit liver and rat kidney complexes and associated kinase was reported subsequently by Harris *et al.* (1982) and Odyssey (1982). The kinase has not been purified but it may be different from pyruvate dehydrogenase kinase (Pettit *et al.* 1978). The amino acid sequence around the phosphorylation site(s) is not known. The kinase may be specific for MgATP; the apparent K_m is 13 μm (Lau *et al.* 1982).

The kinase reaction is inhibited by ADP (competitive with ATP), the branched-chain 2-oxoacids (non-competitive with ATP) and TPP. No consistent effects of NAD+, NADH, CoA, isovaleryl-CoA or acetyl-CoA were detected (Lau *et al.* 1982). In well coupled rat heart or skeletal muscle or kidney mitochondria, inactivation and phosphorylation of branched-chain complex is inhibited by branched-chain ketoacids (Parker & Randle 1978*b*, 1980; Odyssey 1980*b*; Randle *et al.* 1981). In rat heart *in vivo*, or perfused *in vitro* with glucose or pyruvate the proportion of active complex (approximately 8%) is increased by ketoleucine or leucine (which forms ketoleucine) (Parker & Randle 1980; Waymack *et al.* 1980; Harris *et al.* 1982).

All attempts to detect branched-chain 2-oxoacid dehydrogenase phosphatase activity in extracts of mitochondria have so far been unsuccessful. Dephosphorylation and reactivation of phosphorylated rabbit liver complex by a broad specificity phosphatase from rat liver (which may be cytosolic) has been described (Harris *et al.* 1982). Reactivation of inactive complex in mitochondria or rat heart is well documented (Parker & Randle 1978*b*, 1980; Waymack *et al.* 1980; Harris *et al.* 1982).

Tissue-specific regulation; activator protein

Active complex is readily extracted from liver and kidney mitochondria but not from muscle mitochondria. Parker & Randle (1980) could not detect interconvertible active and inactive forms of branched-chain complex in liver and kidney mitochondria. They suggested differential

regulation of the complex in muscles compared with liver and kidney. Subsequent studies with liver mitochondria confirmed their findings but showed that phosphorylation and inactivation could be induced by lowering the osmotic pressure or by exposure to A23187 + EGTA (Hughes & Halestrap 1981; Aftring *et al.* 1982; Patel & Olson 1982). Inactivation was shown subsequently in rat kidney mitochondria by a longer period of incubation but it was less complete than in muscle mitochondria (Odyssey 1980*b*; Lau *et al.* 1981). Other experiments showing differences in ATP inactivation of branched-chain complex in liver and kidney compared with muscles are given in Odyssey (1980*a*), Lau *et al.* (1981), Aftring *et al.* (1982), Waymack *et al.* (1980) and Patel *et al.* (1981). Livesy & Lund (1980) showed that branched-chain 2-oxoacids are released by hindlimbs in the rat and taken up by the liver. These observations suggested that branched-chain complex may be inactivated by phosphorylation in muscles but protected in some way from inactivation in liver.

Fatania *et al.* (1982) have shown that rat liver and rat and ox kidney mitochondria contain a factor that reactivates phosphorylated ox kidney or rat heart branched-chain complex without detectable dephosphorylation. The kinetic properties are those of activation and not of enzymic conversion. The factor has not been detected in heart or skeletal muscle mitochondria. It is thermolabile, inactivated by trypsin and non-diffusible by dialysis and fractionates as a protein or is closely associated with a protein ($M_r \approx 120\,000$ by gel filtration). The most highly purified preparations have a $K_{0.5}$ of approximately 1.2 µg ml^{-1} (Fatania *et al.* 1982; Randle *et al.* 1983). It is not known whether activator protein has other actions (e.g. whether it is a kinase inhibitor) or whether it is itself regulated.

BIOLOGICAL SIGNIFICANCE OF REVERSIBLE PHOSPHORYLATION OF BRANCHED-CHAIN COMPLEX

Current evidence reviewed here suggests that branched-chain amino acids are transaminated to branched-chain 2-oxoacids in extrahepatic tissues and that degradation may be hepatic and extrahepatic. If, as current evidence suggests, only approximately 10 % of branched-chain complex in muscles is in the active form, degradation may be predominantly hepatic. Activator protein in liver could be an important factor in achieving predominantly hepatic degradation but it has yet to be shown that activator protein is physiologically important. Interconversion in muscles may provide a variable pool of active complex, adjusting rates of degradation of branched-chain amino acids to supply. It is possible that predominantly hepatic degradation may be related to the role of the liver in forming glucose and ketone bodies from branched-chain 2-oxoacids.

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