Invertebrate Facultative Anaerobiosis

A reinterpretation of invertebrate enzyme pathways suggests new approaches to helminth chemotherapy.

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Because of the use of classical laboratory animals for most biochemical studies, the degree to which highly successful metazoan organisms utilize anaerobic mechanisms to sustain temporary or indefinite periods of anoxia is not widely appreciated. Nevertheless, it is now clear that many invertebrates are true facultative anaerobes, capable of surviving indefinitely in the absence of oxygen and capable of active oxidative metabolism in its presence. In these organisms, as in primeval ones that arose under reducing conditions in the absence of molecular oxygen (*1*), cellular work is linked to substrate-level phosphorylations such as those occurring in glycolysis, where organic substrates, instead of oxygen, are the acceptors of electrons and protons. Examples of facultative anaerobes are particularly common among widely diversified helminths (*2, 3*); however, during recent years it has also become evident that many mollusks have comparable metabolic capacities, and reaction mechanisms have now been well described (*4, 5*). From the obligate helminth anaerobes to the more versatile facultative anaerobes such as the oyster and other intertidal bivalves, a complete range of anaerobic mechanisms are to be found. The principles of facultative anaerobic metabolism can be well illustrated by considering the data obtained from studies of the adductor muscle of intertidal bivalves such as *Crassostrea gigas* and should be applicable to all invertebrate facultative anaerobes.

The adductor muscle of intertidal bivalves stores remarkably high concentrations of glycogen. Under aerobic conditions the catabolism of glycogen leads to pyruvate, which is then fully oxidized to carbon dioxide and water by the Krebs cycle reactions (*4*), a metabolic organization not unlike that occurring in vertebrate muscle.

Under anoxic conditions, the breakdown of glycogen (glucose) to the level of phosphoenolpyruvate (PEP) is also similar to the process in vertebrates, but in contrast to vertebrates (which convert PEP to pyruvate and accumulate lactate), the main end products of anaerobic glucose catabolism in intertidal bivalves are succinate and alanine (*4–7*). Succinate also accumulates in parasitic helminths such as *Ascaris lumbricoides* (*3*). In all these forms, the production of succinate in muscle during anoxia is apparently directly proportional to the work performed (*3*); that is to say, in these organisms the metabolic production of succinate is the major energy-yielding mechanism during anoxia.

Although the metabolic pathways leading from PEP to succinate have been in dispute (*4, 5*), current evidence strongly indicates that during the aerobic-anaerobic transition, pyruvate kinase activity (E.C. 2.7.1.40) is reduced, while PEP carboxylation to oxaloacetate is favored. The latter reaction is catalyzed by PEP carboxykinase (E.C. 4.1.1.32) and the newly formed oxaloacetate is subsequently converted to succinate (*5–7*).

In helminths such as the adult *Ascaris*, which is in effect an obligate anaerobe, this pathway is strongly favored by the absence of pyruvate kinase and lactate dehydrogenase (E.C. 1.1.1.27) (*3*). However, in intertidal bivalves, the aerobic-anaerobic transition may occur over time periods that are as short as a single tide-cycle (*8*). These organisms maintain both pyruvate kinase and PEP carboxykinase activity (*8–10*). Both enzymes occur in the soluble fraction of the cell and consequently both are competing for the same PEP pool during aerobic-anaerobic transition. The question that arises is what controls the flow of PEP toward pyruvate during aerobiosis but toward oxaloacetate during anoxia.

Control of the PEP Metabolic Branchpoint

From the demonstrable properties of pyruvate kinase and PEP carboxykinase, it appears that the two enzymes are not able to function at significant rates simultaneously; rather, their catalytic requirements and control properties appear to be arranged for function on a reciprocal basis (Table 1). We have previously marshaled the information leading to this conclusion (*9, 10*), and our argument can be summarized as follows.

The activities of many enzymes that participate in cellular energy metabolism are governed at least in part by the energy status of the cell. The usual indicators of the metabolic state are the adenylates: high concentrations of adenosine triphosphate (ATP) are often inhibitory to the enzymes involved in energy metabolism. On the other hand, high concentrations of adenosine monophosphate (AMP) (equivalent to low concentrations of ATP) are often stimulatory (*11*). In oyster muscle, both the pyruvate kinase–catalyzed transphosphorylation reaction and the PEP carboxykinase–catalyzed reaction generate a high-energy phosphate compound (ATP in the case of pyruvate kinase; inosine triphosphate or guanosine triphosphate in the case of PEP carboxykinase) and both are subject to inhibition by the products of their reactions (*9, 10*). In this sense, both enzymes behave in accordance with Atkinson's concept of energy charge (*11*). Whereas these mechanisms undoubtedly contribute to the physiological degree to which PEP is utilized by either reaction pathway, they do not, of themselves, display adequate specificity to account for transition from aerobic to anaerobic metabolism. The specificity required can be supplied by H^+ and L-alanine.

It is widely held that under anaerobic conditions, molluscan bivalves sustain substantial acidification of their tissues and fluids (*12*). This drop in *p*H appears to us to play a pivotal role in the channeling of PEP away from the pyruvate

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kinase reaction and toward PEP carboxykinase, because the *p*H profiles for the PEP carboxykinase and pyruvate kinase reactions are essentially nonoverlapping (*9, 10*). In consequence, in the absence of any other factor, a decreasing *p*H leads to an automatic inhibition of pyruvate kinase with a concomitant activation of PEP carboxykinase. At the same time L-alanine, which accumulates along with succinate under anaerobic conditions, potently inhibits pyruvate kinase (by increasing the Michaelis constant, $K_{m(PEP)}$, and decreasing the maximum catalytic rate). It is particularly instructive that the L-alanine inhibition is potentiated by decreasing *p*H; for pyruvate kinase in the adductor muscle, the inhibition constant, $K_{i(alanine)}$, at *p*H 7.5 is only one-sixth of the K_i value observed at the optimal *p*H of 8.5 (*9*). Indeed, low *p*H likewise potentiates the inhibition of pyruvate kinase by ATP (*9*). In sharp contrast, the primary effects of L-alanine on PEP carboxykinase appear to be (i) a reversal of inosine triphosphate inhibition and (ii) a slight activation at low PEP concentrations due to a reduction in the apparent $K_{m(PEP)}$ (*10*). Both these effects of L-alanine on PEP carboxykinase occur at *p*H ranges in which pyruvate kinase activity is very low and in which L-alanine and ATP inhibition of pyruvate kinase is unusually extreme. The net effect of decreasing *p*H and increasing L-alanine concentration is an autocatalytic increase in the PEP carboxykinase activity concurrent with an exponential decrease in the pyruvate kinase activity (Fig. 1). This would appear to be an adequate arrangement for channeling PEP toward oxaloacetate.

Table 1. Properties of pyruvate kinase and phosphoenolpyruvate carboxykinase in the oyster. [Data from Mustafa and Hochachka (*9*) and Mustafa (*10*)]

Characteristic	Pyruvate kinase	PEP carboxykinase
*p*H optimum	7.5–8.5	5.0–6.0
Ion requirement	Mg^{2+}, Mn^{2+}	Zn^{2+}
Cosubstrate	ADP	IDP, GDP
Minimum $K_{m(PEP)}$	0.09 m*M*	0.18 m*M*
Negative modulators	ATP, alanine, H^+	ITP, GTP, OH^-
Positive modulators	FDP, OH^-	Alanine, H^+
FDP reversal of ATP and ITP inhibition	+	–
Alanine reversal of ATP and ITP inhibition	–	+

The Major Metabolic Fate of Cytoplasmic Oxaloacetate

Most of the oxaloacetate formed from PEP carboxylation is reduced to malate. This appears to be true both in molluscs and in helminths (*3, 6, 13*). In tissues of these organisms, the activity of cytoplasmic malate dehydrogenase (E.C. 1.1.1.37) is high, usually much higher than either PEP carboxykinase or pyruvate kinase activities. The equilibrium position for the malate dehydrogenase reaction is far in the direction of malate, and it is therefore widely accepted that the enzyme functions (i) in the maintenance of low oxaloacetate concentrations thus preventing significant reversal of PEP carboxykinase, and (ii) in regenerating nicotinamide adenine dinucleotide (NAD) for the glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12) reaction (*13*). If all the carbon of glucose is diverted into this pathway during anaerobiosis (that is, if pyruvate kinase is fully blocked), for each two reducing equivalents formed by the glyceraldehyde-3-phosphate dehydrogenase reaction, two are oxidized by the malate dehydrogenase reaction, and to this point the system is in perfect redox balance. It will be recognized that lactate dehydrogenase serves an identical function in anaerobic glycolysis of vertebrate tissues.

The Metabolic Fate of Malate

There are two alternative routes for the metabolism of malate, and from available data it appears that each route competes approximately equally for cytoplasmic malate. In one reaction scheme, malate is converted to fumarate by a reversal of the well-known fumarase reaction (E.C. 4.2.1.2). In bivalves, the fumarase appears to be localized in the cytosol (*5*), but in helminths it is in the mitochondria (*13*). The fumarate in turn is reduced to succinate, by reversal of succinate dehydrogenase (E.C. 1.3.99.1). In helminths and bivalves (*3, 13*), this enzyme appears to be kinetically adapted for function in the direction of succinate production. All previous workers in this field have assumed that this pathway is the major source of the succinate that accumulates during anaerobiosis (*2–4, 13*). As we shall show, it is only one of at least two available routes.

A second route of malate metabolism also begins in the cytosol, for in bivalves malic enzyme [E.C. 1.1.1.40; malate dehydrogenase-(decarboxylating)-NADP] competes directly for cytoplasmic malate. The reaction

$$\text{malate} + \text{NADP} \rightleftarrows \text{NADPH} + \text{CO}_2 + \text{pyruvate}$$

where NADP is the monophosphate of NAD and NADPH is the reduced form of NADP, is catalyzed by malic enzyme and is freely reversible; for this reason its function in anaerobiosis has recently been in dispute (*4*). In the oyster adductor muscle, as in other organisms, malic enzyme is fully reversible; however, the Michaelis constants for pyruvate and carbon dioxide are so high (*10*) that in vivo the oyster enzyme probably functions only in the direction of pyruvate production, and this is also the established direction of function in *Ascaris* (*13*).

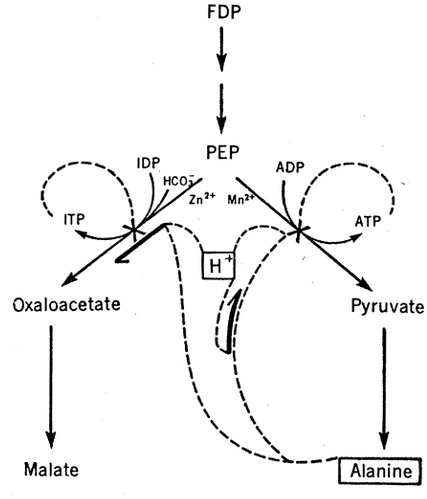


Fig. 1. Known control interactions at the PEP branchpoint in adductor muscle of *Crassostrea gigas*. Effective activation or de-inhibition is shown as a dark arrow; effective inhibition, by a dark cross. In addition to the regulatory interactions shown, fructose diphosphate (*FDP*) is an established feed-forward activator of pyruvate kinase (*9*), and this mechanism could maintain pyruvate kinase activity during anoxia. [Data from Mustafa and Hochachka (*9*) and Mustafa (*10*)]

Metabolic Fate of Pyruvate and a Second Route to Succinate

Whatever the predominant route of pyruvate formation during anaerobiosis, it is probable that the primary metabolic fate of pyruvate is conversion to alanine, a reaction catalyzed by alanine aminotransferase (E.C. 2.6.1.2):

pyruvate + glutamate → α-ketoglutarate + alanine

On this, the evidence in helminths is unequivocal (*2*) and the situation in mollusks appears comparable (*4*). Since bivalves and many helminths possess the enzymes capable of converting α-ketoglutarate to succinate (*2, 4*), this reaction span represents another major pathway for the accumulation of succinate during anaerobiosis (Fig. 2). This pathway may be particularly important because a variety of amino acids could participate in it by way of transamination reactions:

amino acid + α-ketoglutarate → glutamate + keto acid

In most organisms, α-ketoglutarate produced during glutamate-pyruvate transamination is reconverted to glutamate by glutamate dehydrogenase (E.C. 1.4.1.4.), a reaction that utilizes NADH. In faculative anaerobes, the specific activity of glutamate dehydrogenase is very low (*14*), while α-ketoglutarate dehydrogenase (E.C. 1.2.4.2) activities are often high (*3*). For these reasons the α-ketoglutarate dehydrogenase reaction would probably outcompete the glutamate dehydrogenase reaction for the common substrate, α-ketoglutarate. Under these conditions, the alanine aminotransferase serves to channel α-ketoglutarate directly toward α-ketoglutarate dehydrogenase.

Functional Significance of Two Routes to Succinate

The facultative anaerobe gains a critical energetic advantage by utilizing the α-ketoglutarate dehydrogenase reaction pathway. This reaction

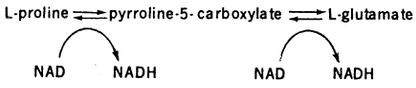
α-ketoglutarate + CoASH + NAD → succinyl $SCoA_1$ + CO_2 + NADH

where CoASH is reduced coenzyme A, sets the stage for conversion of thiolester bond energy into nucleoside triphosphate. The reaction, catalyzed by succinic thiokinase (E.C. 6.2.1.4), is exergonic and can utilize either guanosine diphosphate or inosine diphosphate (GDP or IDP) as cosubstrate, generating guanosine triphosphate or inosine triphosphate (GTP or ITP). This energy-yielding reaction, an integral substrate-level phosphorylation step in the Krebs cycle, is utilized as an anaerobic mechanism for supplanting aerobic metabolism in certain mammalian tissues (*15*), and we presume it has been selected for an analogous function in facultative anaerobic invertebrates. In both systems, however, some provision must be made for the regeneration of the NAD required for α-ketoglutarate dehydrogenase activity. In facultative anaerobes the most likely candidate for this job is succinate dehydrogenase (E.C. 1.3.99.1), which couples the oxidation of NADH (possibly through an intermediate flavin) with the reduction of fumarate to succinate (*2–4, 13*). We propose that it is for this function that the fumarate → succinate reduction was selected in facultative anaerobes. It is this function that explains the unique kinetic features of the enzyme (a relatively high affinity for fumarate and a relatively low affinity for succinate) as well as its high activity in these organisms (*4, 13*). In addition, succinate dehydrogenase is properly positioned in the mitochondrion for the delivery of NAD to the α-ketoglutarate dehydrogenase reaction (*2, 3, 5, 13*).

From these considerations, one can view the unique pathways of anaerobic glucose metabolism in facultative anaerobes as a means for priming the flow of glutamate → α-ketoglutarate → succinyl CoA → succinate, by (i) supplying pyruvate for the alanine aminotransferase reaction and (ii) regenerating NAD through fumarate reduction for the α-ketoglutarate dehydrogenase reaction (Fig. 2). Since the ultimate source of α-ketoglutarate is glutamate, provision must be made for maintaining the glutamate reserves if these mechanisms are to sustain the organism indefinitely.

Potential Metabolic Sources of Glutamate

Up to this point our discussion has been based on experimental data. In attempting to identify major metabolic sources of glutamate, however, we are by necessity more speculative. Nevertheless, it is an old observation that bivalves, as well as many other molluscs, maintain very high tissue concentrations of various amino acids (*16*). Arginine and proline, occurring in concentrations of up to 1 milligram per gram of wet weight of tissue (*16*) appear particularly relevant, for arginine can give rise to proline and the transformation of proline to glutamate is a simple process. Proline is oxidized by a flavoprotein to pyrroline-5-carboxylate. The pyrroline-5-carboxylate is directly oxidized to yield glutamate in a freely reversible reaction:



The relevant reactions have been demonstrated in molluscan tissues (*14*) and an identical function for proline has been described in insects. In some insects, proline is an important carbon and energy source, because it, but not glutamate, can penetrate muscle mitochondria, where it undergoes oxidation to form pyrroline-5-carboxylate and ultimately glutamate (*17*). Glutamate, in turn, feeds into the Krebs cycle by way of the glutamate dehydrogenase reaction. The NAD required for these reactions in the mitochondria may be regenerated by intramitochondrial malate dehydrogenase (*15*). This situation is shown in Fig. 2, but it should be clear that available data do not allow unequivocal assessment of the redox potentials at these loci in metabolism.

Whatever the predominant routes for glutamate production in the facultative anaerobe, the available evidence suggests that it is converted to α-ketoglutarate by way of alanine aminotransferase. Now it is evident that α-ketoglutarate could also be produced from glutamate by the direct action of glutamate dehydrogenase, and the question of the relative roles of these two pathways for producing α-ketoglutarate must be briefly considered. The clue to this problem may be found in the equilibrium positions for the two reactions. In the case of glutamate dehydrogenase, the equilibrium position is far in the direction of glutamate. Hence, to achieve net catalytic function in the direction of α-ketoglutarate, a large thermodynamic barrier must be overcome. Moreover, since H^+ is a kind of substrate for the glutamate dehydrogenase reaction, it may likewise contribute to α-ketoglutarate conversion to glutamate. For these reasons, the glutamate dehydrogenase reaction would not be an efficient source of α-ketoglutarate for catabolism under anaerobic conditions in the bivalve. Unless α-ketoglutarate concentrations are kept low, the glutamate dehydrogenase reaction indeed may channel some α-ketoglutarate toward glutamate. Because of these

properties, it may not be coincidental that glutamate dehydrogenase occurs in only low concentrations in molluscan tissues (*14*).

In contrast, the equilibrium constant for most transaminases is approximately unity. Under these conditions, the direction of net catalytic function is determined by the relative affinities of the enzyme for substrates and products. Thus, in bivalves, alanine aminotransferase seems to have an unusually high affinity for pyruvate and an unusually low affinity for alanine (*4, 18*). These are precisely the conditions under which net synthesis of large amounts of alanine and α-ketoglutarate would be expected and indeed are found in nature.

Metabolic Fate of Alanine and Succinate

Finally, the metabolic fate of the two end products of anaerobic metabolism in bivalves should also be considered. From available data on intertidal bivalves, alanine and succinate appear to accumulate and, if anoxia is sustained indefinitely, they are excreted. Since both metabolites are excellent substrates for aerobic cellular respiration, it is not surprising that, on return to aerobiosis, both metabolites are so actively respired that the bivalve can display an oxygen debt phenomenon (*19*).

The Yield of High-Energy Phosphate Compounds

Although the peculiar nature of anaerobic metabolism in bivalve mollusks has been recognized, little attention has been given to its functional aspects. From our considerations it appears that by utilizing metabolic schemes leading to succinate rather than lactate the bivalve gains a distinct energetic advantage over other organisms that rely solely upon glycolysis for anaerobic energy production. In the classical glycolytic scheme, for 1 mole of glucose metabolized, a net gain of 2 moles of ATP is obtained. In the facultative anaerobe, for 1 mole of glucose + glutamate metabolized, a net gain of at least 3 moles of high-energy nucleoside triphosphate can be generated, if we assume that an obligate redox coupling occurs between succinate dehydrogenase and α-ketoglutarate dehydrogenase. If these are uncoupled, the yield from the α-ketoglutarate pathway can be increased.

Known energy-yielding reactions are catalyzed by (i) phosphoglycerate kinase, (ii) PEP carboxykinase, (iii) pyruvate kinase, and (iv) succinic thiokinase. Cohen (*15*) has calculated that in terms of high-energy phosphate yield the succinic thiokinase pathway by itself is about 50 percent as efficient as glycolysis in the mammalian kidney, and it would appear no less important in the facultative anaerobiosis of invertebrates.

Parenthetically, it should be stressed that certain helminths (for example, *Fasciola hepatica*) accumulate propionate rather than succinate (*19*). In these organisms, succinyl CoA initiates the reaction pathway leading to propionate:

$$\text{succinyl CoA} \rightleftharpoons \text{methylmalonyl CoA} \quad (1)$$

$$\text{methylmalonyl CoA} + \text{ADP} + P_i \rightleftharpoons \text{propionyl CoA} + \text{ATP} + CO_2 \quad (2)$$

$$\text{propionyl CoA} + \text{AMP} + PP_i \rightleftharpoons \text{propionate} + \text{ATP} \quad (3)$$

where PP_i is pyrophosphate. Reaction 1 is catalyzed by methylmalonyl CoA mutase (E.C. 5.4.99.2); reaction 2, by propionyl CoA carboxylase (E.C. 6.4.1.3); and reaction 3 by acetic thiokinase (E.C. 6.2.1.1). It will be evident that stoichiometric coupling of this reaction scheme to glycolysis doubles the high-energy phosphate yield. However, at this time it is not known whether or not bivalve mollusks utilize this mechanism during anoxic excursions.

On the Origins of the Krebs Cycle

In some helminths such as *Ascaris lumbricoides*, pyruvate has a different metabolic fate, accumulating ultimately as acetate (*2, 13*). If pyruvate dehydrogenase is the first step in the reaction pathway, a situation that appears probable (*2, 3*), the reaction scheme

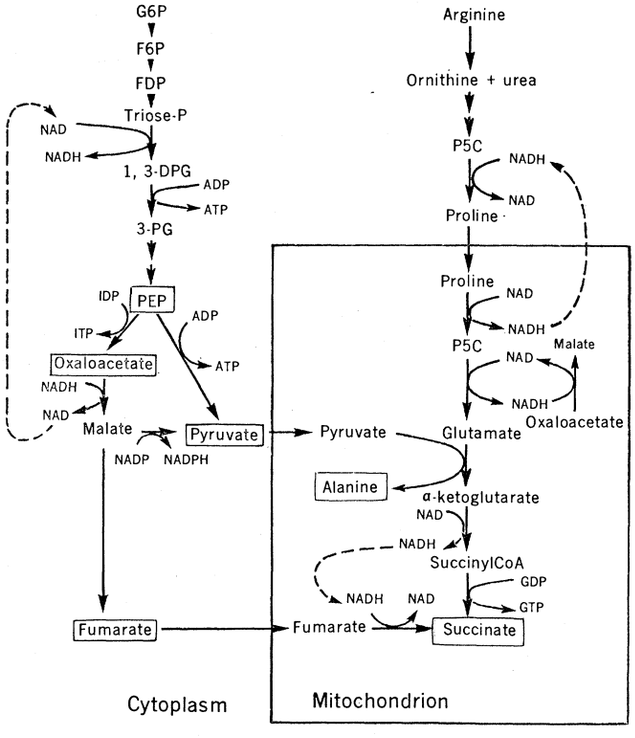
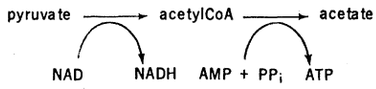


Fig. 2. Probable pathways of anaerobic intermediary metabolism in invertebrate facultative anaerobes. The cellular localization of the fumarase and malic enzyme (*5, 13*) and of the proline pathway may vary between species (*17*). Alanine aminotransferase is seen to play a pivotal role in coupling the simultaneous mobilization of carbohydrates and amino acids; one substrate, pyruvate, arises from glucose, while the second, glutamate, arises from the amino acid pool. Another important mechanism for integrating glucose and glutamate catabolism is the redox couple that forms between α-ketoglutarate dehydrogenase and fumarate reductase. According to this metabolic scheme, half the carbon of glucose should appear in alanine, half in succinate; this has been shown (*6*). Also, half the succinate should arise from glucose, while half should arise from glutamate. This prediction has not been tested. However, the corollary that, after the administration of ^{14}C-labeled glucose, the specific activity of succinate should be half that of alanine has been established (*8*). As drawn, the scheme is not in redox balance. NADPH produced by malic enzyme may be reoxidized by reductive biosyntheses, such as fatty acid synthesis. However, available data do not allow a complete assessment of this problem. Symbols: *G6P*, glucose-6-phosphate; *F6P*, frutose-6-phosphate; *FDP*, fructose diphosphate; *1,3-DPG*, 1,3-diphosphoglycerate; *3-PG*, 3-phosphoglycerate; *P5C*, pyrroline-5-carboxylate.

catalyzed by pyruvate dehydrogenase and acetic thiokinase is formally analogous to that for the metabolism of α-ketoglutarate:



This route, therefore, supplies the organism with another potential mechanism for the anaerobic generation of ATP. As in the case of α-ketoglutarate dehydrogenase, the NAD for pyruvate dehydrogenase may be regenerated by succinate dehydrogenase. This reaction pathway would probably be favored in those facultative anaerobes that rely solely upon glucose as a carbon and energy source. In contrast, in the bivalve molluscs, concentrations of free amino acids are up to 100 times greater than those occurring in mammalian tissues (see *14, 16*), and these are widely recognized as important potential energy sources. In these groups, the α-ketoglutarate pathway is probably favored because it couples glucose and amino acid catabolism. Indeed, it is the ready availability of amino acids that may have allowed the selection of an alternative role for pyruvate dehydrogenase: the generation of acetyl CoA for condensation with oxaloacetate to form citrate.

This last statement is tantamount to suggesting a significant anaerobic function for the first span of the Krebs cycle—the span catalyzed by citrate synthase (E.C. 4.1.3.7), aconitase (E.C. 4.2.1.3) and isocitrate dehydrogenase (E.C. 1.1.1.41). The third reaction in this span generates α-ketoglutarate, and if our considerations are correct, any anaerobic scheme which produced α-ketoglutarate in these organisms would be strongly selected since it sets the stage for an efficient substrate-level phosphorylation at a time when high-energy phosphate compounds are at a premium.

The detailed exploration of this question is beyond the scope of this article (*20*). It is sufficient to say that with the elaboration of enzymes catalyzing the first span of the Krebs cycle, all the enzymes of that cycle were available for serving anaerobic functions. These are precisely the conditions that would be required to explain the origin of an aerobic metabolic scheme as complex as that of the Krebs cycle. With these conditions satisfied, the subsequent utilization of oxygen as a terminal electron acceptor released these reactions from their anaerobic functions; they could now be organized into a different sequence to subserve a different physiology.

Potential Medical Implications

Our reinterpretation of the nature of energy metabolism in invertebrate facultative anaerobes opens up some new horizons for chemotherapeutic approaches to the control of helminth parasites in man and domestic animals. An obvious and basic strategy here is one that aims at an enzyme reaction which is either (i) entirely unique to the helminth and is not at all operative in the host, or (ii) displays especial regulatory and catalytic requirements in the parasite. In this context, PEP carboxykinase is an obvious candidate for chemotherapeutic attack. Thus, in the facultative anaerobe, the enzyme plays a specific role in energy metabolism; in the host, its function is biosynthetic. In the anaerobe, it functions in the carboxylation direction; in the host, the enzyme functions in the decarboxylation of oxaloacetate. In the former, PEP carboxykinase shows an acid *p*H optimum; in the host, it shows an alkaline *p*H optimum. In the anaerobe, the enzyme displays an absolute requirement for Zn^{2+}; in the host, it shows an absolute requirement for Mn^{2+} or Mg^{2+} (see *9, 10, 13*). Any of these characteristics that are absent in the host enzyme might be utilized in the chemotherapeutic control of the parasite.

Conclusions

The unique pattern of anaerobic carbohydrate metabolism in invertebrate facultative anaerobes *serves to couple other substrate-level phosphorylations to the glycolytic reactions, thus increasing the potential yield of high-energy phosphate compounds*. Currently, two important coupling sites can be identified:

1) Succinate dehydrogenase catalyzes fumarate reduction to succinate. The reaction ultimately regenerates NAD, and thus supplies coenzyme for α-ketoglutarate dehydrogenase.

2) Alanine aminotransferase catalyzes the formation of alanine and α-ketoglutarate, and thus supplies substrate for α-ketoglutarate dehydrogenase. Succinyl CoA formed by the α-ketoglutarate dehydrogenase reaction can be utilized to "drive" the substrate-level phosphorylation of GDP (or IDP) to GTP (or ITP).

Cosubstrates for both of the above coupling reactions, fumarate for the first and pyruvate for the second arise from glucose. Hence the two coupling reactions can be viewed as a means for achieving the simultaneous mobilization of glucose and glutamate during anoxic excursions.

This reinterpretation of anaerobic energy metabolism in facultative anaerobes raises the possibility of new chemotherapeutic approaches to the control of helminth parasites in domestic animals and in man.

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