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MINI-REVIEW

Wil N. Konings · Juke S. Lolkema · Bert Poolman

The generation of metabolic energy by solute transport

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Abstract Secondary metabolic-energy-generating systems generate a proton motive force (pmf) or a sodium ion motive force (smf) by a process that involves the action of secondary transporters. The (electro)chemical gradient of the solute(s) is converted into the electrochemical gradient of protons or sodium ions. The most straightforward systems are the excretion systems by which a metabolic end product is excreted out of the cell in symport with protons or sodium ions (energy recycling). Similarly, solutes that were accumulated and stored in the cell under conditions of abundant energy supply may be excreted again in symport with protons when conditions become worse (energy storage). In fermentative bacteria, a proton motive force is generated by fermentation of weak acids, such as malate and citrate. The two components of the pmf, the membrane potential and the pH gradient, are generated in separate steps. The weak acid is taken up by a secondary transporter either in exchange with a fermentation product (precursor/product exchange) or by a uniporter mechanism. In both cases, net negative charge is translocated into the cell, thereby generating a membrane potential. Decarboxylation reactions in the metabolic breakdown of the weak acid consume cytoplasmic protons, thereby generating a pH gradient across the membrane. In this review, several examples of these different types of secondary metabolic energy generation will be discussed.

Key words Metabolic energy · Proton motive force · Secondary transport · Malolactic fermentation · Decarboxylases · End-product efflux · Precursor/product exchange

Abbreviations *pmf* Proton motive force · *smf* Sodium ion motive force

For all living cells, it is essential to generate energy in a form that can be used to drive energy-requiring processes, such as the synthesis of macromolecules, motility, uptake of solutes across the cytoplasmic membrane, etc. This energy is termed 'metabolic energy'. There are in essence two forms of metabolic energy: energy-rich phosphate-bond intermediates, with ATP as the major representative, and electrochemical energy stored in ion gradients, mainly proton or sodium-ion gradients. These two forms of metabolic energy can be obtained by substrate level phosphorylation and by chemiosmotic free-energy-conserving processes (Mitchell 1968). The free energy released in a particular enzymatic reaction is directly used in the process of substrate level phosphorylation for the synthesis of ATP, which functions as a universal molecular carrier of metabolic energy. In chemiosmotic free energy conservation, chemical, light, or redox energy is converted into electrochemical energy by coupling the chemical reactions to vectorial translocation of ions across the membrane. In most bacteria, these ions are protons, but in a number of bacteria, sodium ions are the major coupling ions in energy-transducing processes (Lolkema et al. 1994). The systems that generate these electrochemical ion gradients are the 'primary transport systems', and the process is 'primary metabolic energy generation' (Fig. 1). The electron transfer systems (respiratory chains, anaerobic electron transfer systems, phototrophic light-driven electron transfer systems) and the membrane-bound F_0F_1 -ATPases are the best-studied representatives of primary transport systems. The electrochemical gradient of a particular ion exerts a force on these ions, which is termed the 'ion motive force', i.e., proton motive force (pmf) or sodium ion motive force (smf). The membrane-bound electron transfer systems are powerful pmf- or smf-generating systems, which are mainly responsible for pmf or smf generation in bacteria that can use light or redox en-

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primary

secondary

Fig. 1 Primary and secondary metabolic energy generation in bacteria. The chemiosmotic pumps shown on the *left* and from *top to bottom* are electron transport chains, light driven proton pumps, and F_0F_1 -ATPase, respectively. The secondary systems on the *right* represent the electrogenic uniporters, the proton symporters involved in end-product excretion, and the electrogenic precursor/product exchangers

ergy. Organisms that can only grow fermentatively depend to a large extent on metabolic energy generation by substrate level phosphorylation. The generally accepted view is that in these organisms, the pmf and/or smf is generated by the action of the membrane-bound ATPase. However, recent studies have revealed that primary metabolic energy generation is not the only process by which a pmf or smf can be generated (Otto et al. 1980; Poolman 1990; Konings et al. 1994). In fermentative bacteria, a pmf or smf can be generated by secondary transport processes and, by analogy, these processes are called 'secondary metabolic energy generation' (Fig. 1). The secondary transport systems convert the (electro)chemical energy of a solute gradient into electrochemical energy of proton and sodium ion gradients (Poolman and Konings 1993). The resulting forces can subsequently be used for other energy-requiring processes. In this review, several well-established examples of secondary metabolic energy generating systems will be described.

Energetics of secondary transporters

The proton motive force or sodium ion motive force contributes to the forces involved in the transport step of most secondary transport processes. The ion motive force is composed of two components: an electrical potential across the membrane ($\Delta\Psi$), and a chemical potential of protons ($-Z\Delta pH$) or sodium ions ($-Z\Delta pNa$), according to the following equation:

$$\frac{\Delta\tilde{\mu}_{X^+}}{F} = -\frac{2.3RT}{F}\Delta pX + \Delta\Psi \quad (1)$$

in which X^+ represents either H^+ or Na^+ , and the forces are expressed in electrical field units (V).

Secondary transport systems convert the (electro)-chemical energy of one solute into the (electro)-chemical energy of another solute. They are classified in three groups (Fig. 2): (1) *uniporters* catalyze the translocation of only one solute across the cytoplasmic membrane; (2) *symporters* mediate the coupled transport of two or more solutes in the same direction; and (3) *antiporters* couple the movement of one solute in one direction to the translocation of another solute in the opposite direction. Usually, but not necessarily, one of the solutes in the coupled translocation process is a proton or a sodium ion. The solutes that are transported by the secondary transport systems can be neutral or negatively or positively charged. Furthermore, the number of protons (or sodium ions) translocated per solute can vary. Consequently, the forces that drive the translocation processes differ (see Fig. 2). It should be emphasized that the direction of net translocation is determined by the direction of the driving force, unless other regulatory factors prevent such an action.

In proton-dependent symport and antiport systems, the proton motive force or one of its components is used to drive the accumulation or extrusion of another solute. In this way, a chemical gradient of the solute is generated which, at thermodynamic equilibrium, will be equal and opposite to the driving force supplied by the proton motive force. Thus, at thermodynamic equilibrium, the chemical gradient of A (ΔpA) will become equal, but opposite in sign, to the driving force exerted by the components of the pmf. If for some reason the chemical gradient of A exceeds the driving force supplied by the proton motive force, the reversed process can occur and ΔpA will drive

Fig. 2 The three classes of secondary transporters. The force on the translocation process is indicated on the *right*. By definition, the gradients are defined as 'in' minus 'out' (A solute, z charge of solute, $pA = -\log[A]$, $\Delta\Psi$ membrane potential, ΔpH pH gradient, $Z RT/F$)

the process in the opposite direction, which will lead to the generation of a pmf. Thus, in principle, the activity of secondary transport systems can lead to the generation of a proton motive force, which, subsequently, can drive other metabolic energy-requiring processes, such as ATP synthesis by the membrane-bound F_0F_1 -ATPase. Studies in anaerobic bacteria have revealed that these latter processes are rather common and play an important role in the bioenergetics of these organisms. Substrate uptake and product excretion can contribute to energy conservation in the form of an ion motive force. If the overall process involves the net translocation of charge, the electrical potential across the membrane will be affected; when protons or sodium ions are translocated, the pH gradient or the chemical sodium ion gradient across the membrane will be changed. In several of the processes that will be discussed below, the transport process is followed by metabolism in the cytoplasm of the accumulated solute (precursor). If in this metabolism net protons are consumed, an additional generation of a ΔpH will occur.

Excretion systems

End-product efflux

During the fermentation of a carbon and/or nitrogen source, metabolic intermediates and/or end products of metabolism are produced intracellularly. The internal concentrations of these end products will rapidly exceed the external concentration, and these molecules have to leave the cell, which in many cases is mediated by secondary transport systems. An example of such a situation is found during glucose or lactose fermentation in *Escherichia coli* and *Lactococcus lactis*, respectively. These fermentations lead to the production of lactic acid, which can be excreted via a secondary transport system. The excretion process has been studied in vivo (Otto et al. 1980; Ten Brink and Konings 1982; Ten Brink et al. 1985) and in membrane vesicles (Ten Brink and Konings 1980; Otto et al. 1982) within the pH range 5 to 8. The apparent pK of lactic acid is 3.9, and at high intracellular pH values, essentially all lactic acid is in the anionic form. At high pH values, the excretion process results in the generation of a membrane potential, inside negative. This shows that the process is electrogenic, indicating that more than one proton is translocated per lactate anion. At low pH values, lactate excretion does not generate a membrane potential. The excretion is an electroneutral process and only one proton is translocated per lactate anion. These observations indicate that at high pH values, lactate is excreted by a proton symport system with two or more protons (Konings and Booth 1981), while at low pH values, excretion occurs via the same transport system, but with a proton/lactate stoichiometry of one and/or by passive diffusion of the undissociated lactic acid (Fig. 3). The degradation of glucose by the glycolytic pathway leads to the formation of lactic acid (lactate anion plus one H^+). Therefore, only excretion of lactate in symport with two

Fig. 3 Lactate excretion at low and high internal pH. Lactic acid produced from glycolysis is excreted by passive diffusion across the membrane at low internal pH when a significant fraction of protonated lactic acid (*HLac*) is present and by carrier-mediated transport at high pH as lactate (*Lac⁻*) in symport with two protons. The latter process results in the generation of metabolic energy ('energy recycling')

or more protons will lead to the generation of a pmf and contribute to additional metabolic energy during the fermentation process. The process in which excretion of metabolic end products via ion symport mechanisms results in the generation of metabolic energy has been termed 'energy recycling' (Michels et al. 1979). Evidence has been presented for H^+ -linked carrier-mediated excretion of lactate in *L. lactis* (Otto et al. 1980, 1982), *E. coli* (Ten Brink and Konings 1980) and *Enterococcus faecalis* (Simpson et al. 1983a, b) and Na^+ -linked excretion of succinate in *Selenomonas ruminantium* (Michel and Macy 1990). Excretion of succinate in *E. coli* has been shown to generate a membrane potential (Engel et al. 1994). Carrier-mediated excretion of acetate in *Syntrophomonas wolfei* (McInerney and Beaty 1988), *Desulfovibrio desulfuricans* (McInerney and Beaty 1988), several other sulfate reducers (Ingvorsen et al. 1984; Schönheit et al. 1982), *Acinetobacter woodii* (Boenigk et al. 1989), and some methanogens (Smith and Lequerica 1985) has also been claimed to lead to pmf generation. Furthermore, the excretion of inorganic end products, such as NH_4^+ by *Ureoplasma* species (Kelly 1988) and sulfate by *Thiobacillus* species and other sulfur-oxidizing bacteria (Kelly 1988), may yield additional metabolic energy by mechanisms similar to that proposed for the (weak) acids.

Energy storage systems

A very interesting energy recycling system has recently been found in *Acinetobacter johnsonii* 210A. In the presence of excess carbon and energy source, this organism is able to accumulate large amounts of phosphate together with divalent cations, and these ions are 'stored' in cytoplasmic polyphosphate granules. Phosphate translocation across the cytoplasmic membrane is mediated by a primary binding-protein-dependent uptake system and a proton-motive-force-driven transport system (Van Veen et al. 1993, 1994a). The secondary phosphate-carrier protein has been shown to mediate the uptake and efflux of a soluble, neutral metal-phosphate ($MeHPO_4$) chelate in sym-

Fig. 4 Metabolic energy generation by metal-inorganic phosphate/ H^+ symport. The breakdown of polyphosphate ($Me\text{-}PolyP_n$), stored in granules inside the cell, to the monomeric Me-phosphate complex ($MeHPO_4$) generates an outwardly directed gradient that is used to drive protons outside the cell

port with a proton (Van Veen et al. 1994b). When respiration is impaired in *A. johnsonii* 210A (e.g., in the absence of oxygen or an electron donor), polyphosphate is degraded, and a substantial outwardly directed $MeHPO_4$ gradient (of at least 100 mV) is maintained for several hours. The coupled electrogenic excretion of $MeHPO_4$ and H^+ via the secondary $MeHPO_4$ -transport system has been shown to generate a proton motive force that can drive various metabolic energy-requiring processes, such as the synthesis of ATP via the F_0F_1 H^+ -ATPase and the accumulation of solutes (Van Veen et al. 1994b) (Fig. 4). Thus, the degradation of polyphosphate contributes to the overall production of metabolic energy in *A. johnsonii* 210A through the catalysis of $MeHPO_4/H^+$ efflux. This energy-recycling process enables the organism to survive alternating oxic/anoxic conditions, as encountered in natural habitats and in wastewater treatment plants. $MeHPO_4/H^+$ cotransport has also been demonstrated in *E. coli* (Van Veen et al. 1994c), and energy recycling by the excretion of $MeHPO_4$ may also be a mechanism for the conservation of metabolic energy in polyphosphate-accumulating microorganisms other than *A. johnsonii*.

Fermentation processes

Precursor/product exchange

Several exchange systems in bacteria mediate the net translocation of charge across the cytoplasmic membrane, which results in the generation of a membrane potential. If, in addition, in the conversion of substrate to product a proton is consumed, the fermentation will lead to an increase of the internal pH and, consequently, to the formation of a ΔpH . In this case, the pathway generates a proton motive force consisting of both a membrane potential and pH gradient (Fig. 5). Most known examples of such pathways consist of a precursor/product exchanger in combination with precursor decarboxylation. However, citrate uptake in *L. lactis* has also been proposed to be in exchange with one of the products of citrate metabolism (Hugenholtz et al. 1993). The best-studied examples of

Fig. 5A, B Precursor/product exchange. The membrane potential is generated by translocation of **A** net negative charge into the cell or **B** net positive charge out of the cell. **A** Malolactic fermentation in *Lactococcus lactis* (mal malate, lac lactate). **B** Histidine decarboxylation in *Lactobacillus buchneri* (his histidine, ha histamine)

decarboxylation-driven pathways are oxalate/formate exchange (oxalate fermentation) in *Oxalobacter formigenes* (Anantharam et al. 1989), malate/lactic acid exchange (malolactic fermentation) in *L. lactis* (Poolman et al. 1991), and histidine/histamine exchange in *Lactobacillus buchneri* (Molenaar et al. 1993). These pathways are driven by the decarboxylation reaction ($\Delta G_0' \approx -25$ kJ/mol). The translocation process is driven by the inwardly directed substrate concentration gradient and the outwardly directed product concentration gradient. Both gradients are maintained by the decarboxylation reaction. The membrane potential that is generated is a counteractive force and works against the translocation process. The pH gradient can affect the translocation process through (de)protonation of the substrate and product, which affects the concentration of the particular species that is recognized by the transporter.¹ For instance, monoprotonated citrate ($Hcit^{2-}$) entering the cell may deprotonate in the more alkaline cytoplasm, yielding cit^{3-} , which is not recognized by the transporter. As a result, the substrate is trapped inside the cell. The precursor and product of the decarboxylation reactions are structurally related, and both compete for the same binding site on the carrier. Studies in membrane vesicles have demonstrated that, in fact, the exchangers are proton symporters or uniporters that have been optimized to catalyze exchange rather than unidirectional transport. Those steps in the catalytic mechanism that involve translocation of the enzyme:substrate or enzyme:product complex are catalyzed much

¹ The protonation states of the weak acids are indicated as $Hmal^-$ for monoprotonated malate, H_2mal for fully protonated malate, $Hlac$ for protonated lactate, etc.

Table 1 Electrogenic precursor/product exchange. An antiporter catalyzes the coupled exchange of solutes, whereas an exchanger catalyzes exchange of solutes on top of a uniport or ion-symport mechanism

System	Protein	Organism	Reference
ATP/ADP antiporter	–	<i>Rickettsia prowazekii</i>	Winkler 1976
Aspartate/alanine exchanger ^a	–	<i>Lactobacillus</i> sp.	Higuchi et al. 1993
Histidine/histamine exchanger	–	<i>Lactobacillus buchneri</i>	Molenaar et al. 1993
Lysine/alanine antiporter ^b	LysI	<i>Corynebacterium glutamicum</i>	Bröer and Krämer 1990
Lysine/cadaverine exchanger	CadB	<i>Escherichia coli</i>	Meng and Bennett 1992
Malate/lactate exchanger	MalP	<i>Lactococcus lactis</i>	Poolman et al. 1991
Oxalate/formate antiporter	OxlT	<i>Oxalobacter formigenes</i>	Anantharam et al. 1989
Phenylalanine/phenylethylamine exchanger ^a	–	<i>Lactobacillus buchneri</i>	Unpublished
Putrescine/ornithine antiporter	PotE	<i>Escherichia coli</i>	Kashiwagi et al. 1992
Tyrosine/tyramine exchanger ^a	–	<i>Lactobacillus buchneri</i>	Unpublished

^a On the basis of exchange in intact cells

^b Alanine can be replaced by isoleucine or valine as substrate

more efficiently than the translocation of the unloaded carrier. The cell generates the condition of the inwardly and outwardly directed gradient of the precursor and product, respectively, that promotes the exchange mode of transport. Precursor/product exchange in bacteria has extensively been dealt with in a number of recent reviews (Ambudkar and Rosen 1990; Maloney et al. 1990; Poolman 1990; Poolman and Konings 1993). Table 1 updates the current list of electrogenic bacterial precursor/product exchangers.

Malolactic fermentation

Malolactic fermentation is carried out by some species of the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Pediococcus* (Kunkee 1991). In this pathway, L-malate enters the cells and is subsequently decarboxylated by malolactic enzyme to yield L-lactic acid and carbon dioxide, after which the reaction products leave the cell (Fig. 5A). As a consequence of the pK differences of the carboxylic acid groups on the substrate and products, the pathway results in the alkalization of the medium. It has been shown for *Lactococcus lactis* that L-malate utilization results in the formation of a pmf that is sufficiently high to drive ATP synthesis via the F_0F_1 -ATPase (Poolman et al. 1991).

The decarboxylase catalyzes the conversion of L-malate into L-lactate in two steps. First, L-malate is oxidatively decarboxylated to yield pyruvate, while the electrons are donated to NAD^+ , a cofactor of the enzyme. In the second step, pyruvate, which remains bound to the enzyme, is reduced by NADH to L-lactate. Recently, the structural gene encoding the enzyme of *L. lactis* has been cloned; sequence comparison shows that malolactic enzyme is homologous to the malic enzymes that catalyze the conversion of malate into pyruvate (Anasany et al. 1993). The transport of L-malate has been characterized in artificial membranes prepared from *L. lactis* IL1403 (mal^+) and in isogenic mutants that are defective in either L-malate transport or L-malate decarboxylation (Poolman et al. 1991). Membrane vesicles catalyze unidirectional

uptake of L-malate (either as $Hmal^-$ uniport or mal^{2-}/H^+ symport) and L-malate/L-lactate exchange (either $Hmal^-/Hlac$ or mal^{2-}/lac^-). In both cases, a negative charge is translocated into the vesicle, which results in the formation of a membrane potential (inside negative). L-Malate accumulates inside the vesicles in the presence of a pH gradient because of deprotonation of the transported species in the more alkaline internal milieu. The exchange of L-malate²⁻ for L-lactate⁻ is much more rapid than L-malate²⁻/H⁺ symport, and the former reaction is likely to be most relevant under physiological conditions of malolactic fermentation in *L. lactis*. Since charge compensation in the decarboxylation reaction requires the consumption of a proton, the cytoplasm is alkalinized relative to the outside medium, and a ΔpH is formed. Thus, the free energy of the decarboxylation reaction is converted into a pmf simply by compartmentalization of the malolactic fermentation pathway, i.e., stoichiometric uptake of L-malate²⁻ and excretion of L-lactate⁻ into the medium, combined with decarboxylation in the cytoplasm.

Oxalate decarboxylation

The anaerobic bacterium *Oxalobacter formigenes* obtains metabolic energy from the decarboxylation of oxalate to formate. The mechanism of oxalate uptake and formate excretion has been studied by Maloney and coworkers in proteoliposomes in which the oxalate transport system was reconstituted (Anantharam et al. 1989; Ruan et al. 1992). The transport system was found to catalyze the influx of oxalate²⁻ in exchange for formate⁻. The electrogenicity of the exchange reaction was shown by the effects of a membrane potential on the transport, i.e., a membrane potential inside positive accelerates the exchange, whereas a membrane potential inside negative retards the exchange. The oxalate/formate exchange is very similar to the malate/lactic acid exchange, except that the latter system has also been shown to catalyze an electronegative malate H⁻ uniport (Poolman et al. 1991). It cannot be excluded that a similar reaction, i.e., oxalate uniport, can be facilitated by

the oxalate/formate exchange system of *O. formigenes*. Finally, whereas malate fermentation in the lactic acid bacteria yields a pmf in addition to the metabolic energy formed in the glycolytic and/or arginine deiminase pathway, oxalate decarboxylation serves as the sole source of energy for growth of *O. formigenes*.

Histidine decarboxylation

Lactobacillus buchneri decarboxylates histidine to the biogenic amine histamine, which is excreted into the medium (Molenaar et al. 1993). The pK_A s of the imidazole ring of histidine and histamine are 6.0 and 5.8, respectively, and the protonation state of the side chain of both molecules will have a similar dependency on pH. However, histamine carries one additional positive charge, which makes the histidine/histamine exchange electrogenic, resulting in the net movement of a positive charge to the outside. A ΔpH is formed in a manner similar to that observed for malolactic fermentation and oxalate decarboxylation (Fig. 5B).

Other decarboxylation processes

Biogenic amines, such as cadaverine, putrescine, tyramine, and tryptamine, frequently found in food products, can be formed by decarboxylation of the corresponding amino acids (Gale 1946; Rice and Koehler 1976; Ten Brink et al. 1990). In these pathways, mechanisms similar to malate/lactic acid and histidine/histamine exchange may form part of the decarboxylation pathways and contribute to metabolic energy conservation. It is worthwhile recalling that putrescine can be formed from the decarboxylation of ornithine and also from the conversion of agmatine via the agmatine deiminase pathway, in which agmatine and putrescine are transported via an electroneutral antiport (Driessen et al. 1988). Evidence for putrescine/ornithine exchange as part of the ornithine decarboxylation pathway of *E. coli* has been presented (Kashiwagi et al. 1992). Unfortunately, the expected electrogenicity of ornithine/putrescine exchange has not been addressed adequately. There is also some evidence that *Lactobacillus* sp. are able to convert aspartate into alanine and glutamate into α -aminobutyric acid and gain metabolic energy from these reactions most likely as a result of electronegative exchange in combination with decarboxylation of the substrates (Higuchi et al. 1993).

Finally, *Citrobacter* species are able to grow on malonate²⁻, which is converted intracellularly to acetate. If malonate²⁻ is exchanged with the product acetate⁻, the net inward movement of negative charge will generate a $\Delta\Psi$, while the proton consumption in the reaction will generate a ΔpH (Janssen and Harfoot 1990).

Uniport systems

Studies of malate and citrate metabolism in *Leuconostoc oenos* have revealed the existence of a somewhat different

Fig. 6 Pmf generation by citrate fermentation in *Leuconostoc oenos*. Citrate is taken up in the cell by a uniport mechanism translocating negatively charged H_2cit^- . Metabolism of citrate results in the uptake of H^+ from the internal medium. The end products, acetic acid, acetoin, and carbon dioxide, leave the cells in their uncharged state

mechanism by which secondary metabolic energy may be generated. Malolactic fermentation, in this bacterium that grows at more acidic pH values than for instance *Lactococcus lactis*, proceeds through the uptake of negatively charged mono-protonated $Hmalate^-$ by a uniport mechanism rather than by $Hmalate^-/Hlac^-$ (or mal^{2-}/lac^-) exchange. Lactic acid formed in the proton-consuming decarboxylation reaction leaves the cell by passive diffusion. Energetically, the result is the same, i.e., generation of a membrane potential and pH gradient. Again, the whole pathway is driven by the decarboxylase. The difference is in the transporter that, in the case of the uniport, is only driven by the substrate concentration gradient. Therefore, it is essential that a high solute concentration gradient is maintained by keeping the internal concentration of the solute low by rapid internal metabolism (Salema et al. 1994). The different physiological functions of the malate carriers of *L. lactis* and *Lc. oenos* are reflected in the kinetic properties of the transporters. In *L. lactis*, malate/lactate exchange is catalyzed much more efficiently than unidirectional uptake. In *Lc. oenos*, the opposite is true.

Surprisingly, studies of citrate transport in isolated membrane vesicles of *Lc. oenos* showed the same mechanism as observed for malate uptake (Ramos et al. 1994). The citrate carrier catalyzes uniport of monovalent citrate H_2cit^- , implying that citrate metabolism by *Lc. oenos* is involved in the generation of a membrane potential. Citrate metabolism in citrate-fermenting lactic acid bacteria results in alkalinization of the medium, i.e., protons are consumed in the metabolic breakdown inside the cell and a pH gradient is formed. Therefore, citrate metabolism in *Lc. oenos* is predicted to generate a proton motive force consisting of a membrane potential generated during the electrogenic uptake of H_2cit^- and a pH gradient generated by the consumption of scalar protons in the further degradation of citrate inside the cell (Fig. 6). Studies with resting cells of *Lc. oenos* confirmed the prediction made based on the studies with the membrane vesicles. Addi-

tion of citrate to the cells resulted in the generation of both a membrane potential and a pH gradient independent of F_0F_1 H^+ -ATPase activity.

Concluding remarks

The present review clearly demonstrates that anaerobic bacteria have found ways to gain more metabolic energy from fermentation processes than by substrate level phosphorylation alone. Product excretion, precursor/product exchange, and precursor uptake processes catalyzed by secondary transport systems yield a membrane potential (inside negative) and a pH gradient (inside alkaline) as a result of precursor metabolism. The generated pmf is high enough to drive other metabolic-energy-requiring membrane-bound processes. The metabolic energy generated by the secondary transport processes will, in many cases, be supplementary to the substrate level phosphorylation reactions. In *O. formigenes*, oxalate-fermentation-driven pmf generation is the only source of metabolic energy.

Chemiosmotic proton pumps generate the membrane potential and pH gradient component of the proton motive force in a single step by translocating one or more protons across the cytoplasmic membrane. In fermentation-driven pmf generation, the two components are generated in separate steps: an electrogenic transport step and an alkalinizing metabolic step. Nevertheless, the two events are coupled by the nature of the overall reaction. For instance, the conversion of malate into lactate does not produce or consume protons and is electroneutral when all species involved are in their fully protonated state. If the malate entering the cell is more deprotonated than the lactate leaving the cell, which results in a membrane potential, the cytoplasmic decarboxylation reaction consumes a proton, which results in a pH gradient. The equilibrium pmf values generated by the fermentation-driven pmf-generating systems can be very high (> -400 mV). The physiological pmf, however, is not determined by the thermodynamics of the system, but by the kinetics of pmf formation and consumption, and is known to be much lower than the calculated equilibrium values (Poolman et al. 1991; M. Salema et al., unpublished results). Therefore, the efficiency of energy transduction from the chemical reaction in the pathway to the electrochemical proton gradient is low.

Besides conserving metabolic energy, the fermentation-driven secondary metabolic-energy-generating systems alkalinize the medium. It has long been known that bacteria respond to a lowering of the external pH by increasing the levels of amino acid decarboxylases (Gale 1946). A well-studied example is the *cad* regulon of *E. coli* (Meng and Bennet 1992; Neely et al. 1994). The *cad* regulon is composed of genes encoding a lysine decarboxylase (CadA), a putative lysine/cadaverine exchanger (CadB), and a protein (CadC) that senses acidification of the medium and activates transcription of the *cadAB* operon at low pH values. Although decarboxylation (and proton consumption) takes place in the cytoplasm, the

precursor taken up from the medium is converted into a less acidic product that is excreted into medium. As a result, the medium pH rises. In addition to the pH regulation function, other authors have suggested that the *cad* regulon may function as a supplier of carbon dioxide (Takayama et al. 1994). The mechanism of medium alkalization by decarboxylation reactions is particularly important for fermentative bacteria that produce acidic end products that lower the medium pH as a result of sugar metabolism. Co-metabolism of sugar and substrates that can be decarboxylated may keep the medium pH relatively constant, as shown for glycolysis concomitant with malolactic fermentation in *L. lactis* and other lactic acid bacteria (unpublished results).

The secondary metabolic-energy-generating systems may constitute the early mechanisms of metabolic energy conservation. The decarboxylation-driven pathways as we know them to date do not seem to be involved in anabolism, other than providing the cell with energy. The original function of these pathways, before they became energy transducing, may have been to have some control over the internal pH by affecting the medium pH since the conversion of di- or tricarboxylic acids into monocarboxylic acids alkalinizes the medium. At first the substrates may have crossed the membrane in their uncharged protonated state by passive diffusion, but eventually proteinaceous carriers evolved that speeded up the transport, but still carried out electroneutral transport. These transporters evolved to electrogenic carriers that selected for the deprotonated form of the weak acids. This had two advantages: (1) the deprotonated species is more abundant at the medium pH and (2) free energy is conserved in the form of a pmf. In parallel with the evolution of these carriers, the same type of carriers for other substrates may have evolved that could use the pmf as a driving force.

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