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Stoichiometry of Energy Coupling by Proton-Translocating ATPases: A History of Variability

John J. Tomashek[1] and William S. A. Brusilow[1,2]

One of the central energy-coupling reactions in living systems is the intraconversion of ATP with a transmembrane proton gradient, carried out by proton-translocating F- and V-type ATPases/synthases. These reversible enzymes can hydrolyze ATP and pump protons, or can use the energy of a transmembrane proton gradient to synthesize ATP from ADP and inorganic phosphate. The stoichiometry of these processes (H^+/ATP, or coupling ratio) has been studied in many systems for many years, with no universally agreed upon solution. Recent discoveries concerning the structure of the ATPases, their assembly and the stoichiometry of their numerous subunits, particularly the proton-carrying proteolipid (subunit *c*) of the F_O and V_0 sectors, have shed new light on this question and raise the possibility of variable coupling ratios modulated by variable proteolipid stoichiometries.

KEY WORDS: V, F, A-ATPase; proton/ATP ratio.

INTRODUCTION

Proton-translocating ATPases synthesize most of the ATP in living systems. The past 10 years have seen significant advances in the structural characterization of the enzyme, the observation of a rotational mechanism of conformational coupling, and the confirmation of the binding-change mechanism of catalysis.The field is now at a juncture where theoretical transformations of energy can be considered in the context of the physical structure and mechanism of a very tangible molecular engine.

In this review we consider together all the enzymes of the F-(mitochondrial, plastidic, and bacterial) as well as the V-type and the emerging A-type, because they appear to form a family of related enzymes, which most likely share many structural and mechanistic similarities.

THE CHEMIOSMOTIC HYPOTHESIS

Over 30 years ago, Mitchell (1966) proposed the chemiosmotic hypothesis, setting forth a thermodynamic equivalance and interconvertability between a transmembrane electrochemical gradient of protons (the proton-motive force, pmf, also Δp or $\Delta\mu_{H^+}$) and the chemical potential of ATP in equilibrium with ADP and P_i(ΔG_p or ΔG_{ATP}). The theory defined a minimum $\Delta\mu_{H^+}$ necessary for the synthesis of ATP. Thus early efforts to test the theory included experiments examining the stoichiometry of protons transported to ATP molecules consumed or generated (the H^+/ATP stoichiometry, hereafter the coupling ratio, or *n*). Mitchell determined the coupling ratio was 2 (Mitchell and Moyle, 1969; Moyle and Mitchell, 1973). Other early results (reviewed in Brand, 1977) produced values of 2 to 3 for the coupling ratio, assuming $\Delta G_{ATP} = n\Delta\mu_{H^+}$. It became immediately obvious, however, that the coupling ratio was not necessarily integral, nor necessarily constant (Gräber and Witt, 1976; Azzone *et al.*, 1978a, b, c). This led to a reevaluation of the experimental limitations of the measurement techniques (Ferguson and Sorgato, 1982; Kashket, 1985), and further measurements (*e.g.*, Sorgato *et al.*, 1982), which, taken together, provide convincing evidence that the coupling ratio is variable.

Subsequently, several laboratories attempted kinetic measurements of the coupling ratio by simultaneous measurement of the ATP hydrolysis rate and the proton translocation rate; the ratio of the two rates should yield the

[1] Wayne State University School of Medicine, Department of Biochemistry and Molecular Biology, Detroit, Michigan 48201.
[2] To whom all correspondence should be addressed: e-mail: wbrusilo@wayne.med.edu

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coupling ratio. By this method, a coupling value of 2 was measured for both a V-ATPase (Bennett and Spanswick, 1984) and a F-ATPase (Perlin *et al*., 1986). Both the thermodynamic and later kinetic approaches revealed that the coupling ratio was not constant, but that it varied in proportion to the magnitude of the $\Delta\mu_{H^+}$ (Gräber and Witt, 1976; Maloney, 1983; Krenn *et al*., 1993; Possmayer and Gräber, 1994; Davies *et al*., 1994, 1996). Table I gives our best attempt to catalog published coupling ratios; we apologize to anyone overlooked.

Table I. Coupling Ratios *n*: Reported in the Literature

	References
Mitochondria	
2	Mitchell and Moyle, 1969
2	Thayer and Hinkle, 1973
2.6–2.7	Nicholls and Bernson, 1977
2.9	Rottenberg and Gutman, 1977
3	Alexandre *et al.*, 1978
3	Azzone *et al.*, 1978b
3.2 (3–7)	Holian and Wilson, 1980
3.2–3.8	Sorgato *et al.*, 1982
2.2–2.3	Berry and Hinkle, 1983
3	Scholes and Hinkle, 1984
2–3 (7–9)	Van der Bend *et al.*, 1984
2.2–2.4	Ogawa and Lee, 1984
3	Lemasters, 1984
3	Jensen *et al.*, 1986
Chloroplasts	
4.5 (2.5)	Izawa, 1970
3	Portis and McCarty, 1974
3	McCarty and Portis, 1976
3.31	Portis and McCarty, 1976
2.4	Graber and Witt, 1976
3	Dewey and Hammes, 1981
3	Davenport and McCarty, 1981, 1984
2–5	Lemaire *et al.*, 1985
4	van Walraven *et al.*, 1996
Bacteria	
2	Baccharini-Melandri *et al.*, 1977
2.25	Petty and Jackson, 1979a,b
2	Maloney and Hansen, 1982
3–4.3	Maloney, 1983
3.5	Clark *et al.*, 1983
4.4–9	van Walraven *et al.*, 1986
E. coli	
3	Kashket, 1982, 1983
2.2 and 3.6	Vink *et al.*, 1984
2	Perlin *et al.*, 1986
V-ATPase	
2	Johnson *et al.*, 1982
2	Schmidt and Briskin, 1983a
2	Bennett and Spanswick, 1984

VARIABILITY OF THE COUPLING RATIO

The phenomenon of variable coupling ratio was observed by several laboratories, which showed that as the $\Delta\mu_{H^+}$ decreased, the apparent coupling ratio increased, from a value of 2 to 3 at high $\Delta\mu_{H^+}$, to values >20 at low $\Delta\mu_{H^+}$ (Azzone *et al.*, 1978a,b). This variability required more sophisticated approaches to theoretical chemiosmotic mechanisms. One theory suggested the presence of localized proton domains or "coupling units," such that the $\Delta\mu_{H^+}$ shared by respiratory enzymes and the ATPase was at least partially sequestered from the bulk phases (developed extensively in Westerhoff *et al.*, 1983a,b, 1984a,b). This idea was supported by the observation that the bulk-phase $\Delta\mu_{H^+}$ was little affected whether or not phosphorylation was occurring and, conversely, inhibition of either the respiratory enzymes or the ATPase in a coupled system appeared to have little effect on the $\Delta\mu_{H^+}$, up to a point. However, experiments in hybrid systems, using purified proteins from heterologous sources, successfully coupled light-driven proton pumps to F-type ATP synthases, or proton-pumping pyrophosphatases to V-type ATPases (Pitard *et al*., 1996; Schmidt and Briskin 1993b; Hirata *et al*., 2000). These experiments suggest that if the localized proton domains exist, they are either entirely contained within the enzymes themselves (and remarkably flexible in their ability to interact with one another), or they are a biophysical property of the membrane.

The application of nonequilibrium thermodynamics to the question of coupling also generated new explanations for the variability of coupling ratios, specifically the notion of "slippage" (Baccarini-Melandri *et al*., 1977; Van Dam *et al*., 1980; Pietrobon *et al*., 1983,1986,1987; Pietrobon and Caplan, 1985; Zoratti *et al*., 1986; Luvisetto *et al*., 1987; Läuger, 1991). Slippage results when one of two coupled reactions in a cyclic process proceeds without its counterpart, a process also termed intrinsic uncoupling. Essentially, there is some small but finite rate at which slippage (both proton and/or reaction slippage) occurs relative to the rate of the fully coupled cycle. The coupling ratio, which is assumed to be a fixed quantity intrinsically related to the structure and mechanism of the pump, is modified by a coupling efficiency, q $(0 \leq q \leq 1)$, which reflects the percentage of coupling. On the microscopic level, individual enzymes are "skipping a beat" (*i.e*., slipping), either passing a proton without contributing to ATP synthesis, or hydrolyzing ATP without contributing to proton pumping. On the macroscopic level at which coupling ratios are experimentally determined, the measured ratio is coming out lower or higher than the intrinsic coupling ratio as these microscopic slips are averaged across a population of enzymes.

It is important to note that slip is not merely a membrane leak (another important but separate factor, c.f. Van Dam *et al.*, 1980), but an intrinsic property of the enzyme, and must therefore be related to its mechanism and structure. While a slip may decrease the efficiency of the enzyme in terms of energy conservation, it may allow greater dynamic control of the enzyme over varying ranges of $\Delta\mu_{H^+}$ and ΔG_{ATP}. There is evidence for a slip in F-type enzymes (Baccarini-Melandri *et al.*, 1977; Pietrobon *et al.*, 1983; Van Dam *et al.*, 1980; Zoratti *et al.*, 1986), as well as the V-type enzymes (Moriyama and Nelson, 1988). It is expected that slips have evolved to maximize the function of particular enzymes in particular circumstances. Hence, under natural conditions, V-ATPases are not known to synthesize ATP, nor are mitochondrial or plastidic enzymes known to generate a pmf, although these latter two enzymes have mechanisms, apart from slip, to inhibit ATP hydrolysis and proton pumping (Pullman and Monroy, 1963; Pedersen *et al.*, 1981; Ketcham *et al.*, 1984; Nalin and McCarty, 1984). In significant contrast, bacterial ATPases/synthases appear run the structural gamut between F- and V-type enzymes, and appear designed to operate in both directions, depending upon the needs of the cell (*e.g.*, Yokogama *et al.*, 2000).

Finally, it should be noted that experiments both old and recent have shown that coupling is sensitive to external factors, such as the lipid content of the membrane (e.g. Van der Bend *et al.*, 1984) and, more importantly, the absolute values of the internal and external pH (Gräber and Witt, 1976; Maloney, 1983; Krenn *et al.*, 1993; Possmayer and Gräber, 1994; Davies *et al.*, 1994, 1996; Tomashek, 1997). This latter phenomenon has been attributed to the binding/release of protons from a site(s) that alternates its exposure (and its pK_a) on opposite sides of the membrane, thus accounting for vectorial flow of protons. This "alternating access" theory was among the first proposals for the transport of charged entities across membranes through pumps and porters (Patlak, 1957).

SOME STRUCTURE IS BROUGHT TO ALL THIS THEORY

Early bioenergetic studies had little information regarding structures of the enzymes involved in energy coupling. Then "coupling factors" were identified biochemically and visualized by EM; the *unc*, *atp*, and *VMA* genes encoding ATPase subunits were identified genetically, cloned, and sequenced; various enzymes were purified and disassembled; stoichiometries of the individual subunits were determined; the enzyme was functionally reassembled; and finally enzymes were characterized by extensive kinetic and enzymological assays (reviewed in Senior, 1988; Pedersen and Amzel, 1993; Capaldi *et al.*, 1994; Deckers-Hebestreit and Altendorf, 1996; Boyer, 1997; Stevens and Forgac, 1997; Kane, 1999; Forgac, 1999). In the past 10 years, two significant advances have occurred: a static crystal structure showing an asymmetric arrangement of homologous catalytic subunits (Abrahams *et al.*, 1994) and a dynamic assay showing the rotation of some subunits in relation to other subunits (Noji *et al.*, 1997).

Studies on the composition and structure of these enzymes have revealed a remarkable diversity of subtypes and variations on a common theme (for examples, see Senior, 1988; Stevens and Forgac, 1997; Kakinuma *et al.*, 1999; Müller *et al.*, 1999). All of these enzymes have discrete peripheral membrane sectors (F_1 and V_1) and integral membrane sectors (F_O and V_0).These enzymes all possess three catalytic subunits (β in the F type; A in the V type) and three noncatalytic subunits (α and B, respectively) in their peripheral membrane sector. These subunits are all evolutionarily interrelated; the catalytic subunits all hydrolyze or synthesize NTPs, while the noncatalytic subunits all bind, but do not hydrolyze, adenine nucleotides. These enzymes all possess a set of proteolipid proteins (called the *c* subunits in both F- and V-type enzymes) in their integral membrane sector which transport one proton (or in some cases another cation) per subunit by means of a highly conserved amino acid with a carboxyl side chain. Somehow—and this is the black box where the other subunits and diversity enter in—these two common features are energetically coupled.

Paul Boyer proposed the binding-change mechanism of catalysis for the F_1 (and V_1) sectors of these enzymes (reviewed in Boyer, 1989, 1997; Kasho and Boyer, 1989). This theory proposed that the three catalytic sites are not simultaneously equivalent, but that they are in three distinct conformations—empty, ATP bound, and ADP + P_i bound—and, as the empty site binds a nucleotide, one of the bound sites releases its contents, and the third site undergoes catalysis. The energy for this transformation is found in the binding step; the ΔG of catalysis is essentially zero, within the context of the active site. This theory suggested two hypotheses. First, if a flash photo could be taken of the enzyme, it would be found to have its three catalytic sites in three different states. Second, there would be a physical rotation associated with cycling of the three different sites through the different conformations.

The crystal structure of the bovine F_1 supports the first hypothesis (Abrahams *et al.*, 1994). The three catalytic sites were revealed in three different conformations, as predicted from the binding-change mechanism. The second hypothesis is supported by experiments with the

$\alpha_3\beta_3$ hexamer immobilized on a glass slide and observing through a microscope the ATP-dependent rotation of an actin filament attached to a third subunit (the $F_1\gamma$) (Noji *et al.*, 1997). A later version of this experiment immobilized the F_1 attached to the F_O, with the actin filament attached to the *c* subunit of the F_O (Sambongi *et al.*, 1999).

In the current popular model, the catalytic hexamer drives rotation of a stalk of core subunits attached to an annulus of *c* subunits. As the conserved residues of the *c* subunits are swept past (and interact with) another set of integral membrane subunits (held in a constant position relative to the catalytic hexamer by a second "stator" stalk), the proton-binding site alternates from one side of the membrane to the other, and protons are translocated (Vik and Antonio, 1994; Rastogi and Girvin, 1999; Grabe *et al.*, 2000; Oster *et al.*, 2000). Rotational catalysis implies a direct relationship between the coupling ratio and the ratio of catalytic subunits to *c* subunits, a fact recognized previously by several researchers (Cross and Taiz, 1990). One interesting question, relevant to coupling ratios, that remains unanswered is: are the rotational frequencies of the core stalk and the proposed *c* subunit annulus equal? It is assumed that they are, but there could be a gearlike mechanism that makes them unequal. Inequality of rotational frequencies between the stalk and *c* oligomer (the "gear ratio"?) would, obviously, alter the proportionality of the coupling ratio to the stoichiometric ratio.

STRUCTURE MEETS COUPLING

The recent revelations of structure and mechanism have lent strength to structure- and mechanism-based theories of the coupling ratio. A stoichiometry of three for the catalytic subunit is reasonably uncontested. Thus, for the V-type enzyme, the coupling ratio predicted from the *c* subunit to *A* subunit stoichiometry is 2, assuming 6 proteolipids, which matches the measured values. For F-type enzymes, the predicted value would be 3 or 4, for *c* stoichiometries of 9 or 12, respectively. These values seem high, relative to some experimental results, but are certainly still within the experimental ballpark.

The stoichiometry of the proteolipid, however, has been harder to ascertain because it is (1) small, (2) numerous (at least six per complex), and (3) difficult to work with biochemically because of its hydrophobicity. Experimental estimates for the F-type enzyme have ranged from 8 to 14 (*e.g.*, Foster and Fillingame, 1982; Jones and Fillingame, 1998), with 9 and 12 being favorites due to their divisibility by 3. Of these, 12 has become the preferred guess because of the success of genetically fused multimers of the proteolipid (Jones and Fillingame, 1998) and because of evolutionary comparisons to the V type, which is estimated to have six proteolipids (Arai *et al.*, 1988). Each V_0c is twice the size of a F_Oc and believed to be the result of a gene duplication event in early evolutionary history (Nelson and Nelson, 1989; Gogarten *et al.*, 1992; Nelson, 1992). However, the V-type enzyme has also been found to have multiple isoforms of the proteolipid (Hirata *et al.*, 1997), all essential, and the relative proportions of each are not known. Recent analyses of plant V-ATPases from roots under salt stress also indicate that the levels of *c* subunit vary in response to environmental stress (Low *et al.*, 1996).

A RADICAL PROPOSAL: VARIABLE SUBUNIT STOICHIOMETRY

Uncertainty about the *c* subunit stoichiometry has led us to propose another possible mechanism for altering the coupling ratio of these enzymes, namely, that the stoichiometry of the *c* subunit may vary, both between enzymes from different sources, as well as among enzymes in a population from a single source under different environmental conditions. Variable stoichiometry has numerous implications and interpretations. First, there is the simple and unastonishing conclusion that enzymes from different organisms and organelles might have evolved to incorporate different numbers of *c* subunits. It now appears as if stoichiometries can vary between organisms. The yeast mitochondrial enzyme has been partially crystallized and found to have 10 proteolipid subunits (Stock *et al.*, 1999). In contrast, a plastidic F_O has been visualized by atomic force microscopy and 14 proteolipids have been counted (Müller *et al.*, 2000). Compare these stoichiometries to the coupling ratios estimated for mitochondria versus chloroplasts, as listed in Table I, and it will be noticed that mitochondrial enzymes tend to have $n \leq 3$, while chloroplasts tend to have $n \geq 3$, both in keeping with the trend expected from the different subunit stoichiometries. In contrast to these, bacteria (and *E. coli*, in particular) have values of *n* ranging from 2 to 4.

While the basic unit of the proteolipid is highly conserved, there are clearly certain degrees of structural flexibility among subunits from different sources. Thus there are F-type enzymes with two helix and six helix proteolipids, V-type with four and five helices, and A-type (Archaebacterial) with six helices (for examples, see Hirata *et al.*, 1998; Ruppert *et al.*, 1999; Rahlfs *et al.*, 1999; Yokoyama *et al.*, 2000). Presumably the complement of proteolipid genes have customized to the particular cell or organelle to which they belong. Organellar enzymes

have become highly specialized as synthases (mitochondria and plastids) and proton pumps (V-ATPases). Bacterial enzymes, in contrast, seem to show greater variability, presumably because of their wide variety of environments and the greater range of environmental conditions. Thus, it seems both logical and plausible that bacteria might regulate the proteolipid stoichiometry so as to promote the most favorable coupling ratio for the prevailing environmental conditions.

Our variable *c* stoichiometry hypothesis came from studies on plasmids carrying F_O and F_1 genes and on plasmids carrying just F_O genes. One of the original plasmids constructed to contain all of the genes for the F_O and F_1 subunits, pRGP54, also contained a ribosome-binding site (rbs) mutation preceding *uncE*, the *c* subunit gene (Solomon and Brusilow, 1988). This mutation led to a two- to threefold decrease in synthesis of the *c* subunit. Toeprint studies on ribosome-binding sites in the *unc* operon showed that the mutation resulted in a corresponding two- to threefold decrease in ribosome binding (Schaefer *et al.*, 1989). Nevertheless, this plasmid was capable of restoring both ATPase and ATP synthase activities to a strain deleted for all the chromosomal ATPase genes. An identical plasmid, pWSB30.0, with the wild-type rbs, altered the properties of the resulting ATPase. The deletion strain carrying pWSB30.0 regained membrane-bound ATPase activity that was capable of coupling ATP hydrolysis to proton pumping, but the ATP synthase activity was significantly lower than that in the same strain carrying pRPG54. A deletion strain carrying pWSB30.0 was unable to grow on minimal succinate medium. In this multicopy system, the wild-type expression of the *c* subunit produced higher ATPase activity and lower ATP synthase activity than seen in cells carrying the plasmid with the rbs mutation. The only difference between these two plasmids is how well the *c* subunit is synthesized.

Two F_O plasmids differing only in the *uncE* rbs produced very different phenotypes when transformed into the deletion strain. Cells carrying the F_O plasmid with the wild-type rbs grew very poorly compared to cells carrying the F_O plasmid with the mutant rbs (Brusilow, 1987). These studies on F_1F_O plasmids and on F_O plasmids suggested that a change in *uncE* expression also changed the structure of the F_O, and that the activity and function were sensitive to changes in *c* stoichiometry (Solomon and Brusilow, 1988).

More recently, studies were conducted which attempted to quantitate the relative amounts of *c* actually assembled in F_1F_O complexes with different biochemical characteristics (Schemidt *et al.*, 1995). The relative amounts of the *c* subunit were measured in F_1F_O purified from cells carrying either pRPG54 (the low-*c* ATPase plasmid) or pWSB30.0 (the high-*c* ATPase plasmid). The criterion for assembled F_O subunits, specifically assembled *c* subunits, was coimmunoprecipitated with F_1 subunits, using antibody to the F_1. Purified F_1F_O was precipitated with anti-F_1, and the precipitates were then immunoblotted with antibody to F_1F_O. The individual subunits were quantitated by densitometry. These procedures did not determine the absolute stoichiometries of any subunits, but did determine the relative amounts of the *c* subunit present in each preparation. These experiments showed that F_1F_O purified from cells carrying the high-*c* ATPase plasmid contained three to five times as much *c* subunit as F_1F_O purified from cells carrying the low-*c* ATPase plasmid. These results demonstrated that the F_1F_O, as purifed by the standard, published procedure (Foster and Fillingame, 1979), could contain different amounts of the *c* subunit, depending on the expression of *uncE* gene. The large difference in relative stoichiometry was surprising, but was similar to the differences in both gene expression and ribosome binding caused by the mutation in the rbs.

The previous work (Solomon and Brusilow, 1988) showed that higher *c* stoichiometry correlated with higher ATPase activity and lower ATP synthase activity. Lower *c* stoichiometry correlated with lower ATPase activity and higher ATP synthase activity. Our laboratory, therefore, addressed the issue of whether or not the expression of *uncE* relative to other F_O genes could change *in vivo* and if such changes manifested themselves in F_1F_O complexes with altered *c* stoichiometries.

Using in-frame *lacZ* fusions to measure the translation rates of *uncB* (the *a* subunit gene) and *uncE*, it was shown that *E. coli* changed the relative expression of these two genes when grown on fermentable (glucose) versus a nonfermentable (succinate) carbon source (Schemidt *et al.*, 1998). During growth on succinate, the ATPase must act as an ATP synthase, since the cell has no substrate-level phosphorylation process capable of generating net ATP synthesis from succinate. In cells grown on succinate, the expression of *uncB* was 10–20% higher than in cells grown on glucose at all cell densities tested. The same was true for overall transcription rates of the operon, which were slightly higher during growth on succinate. Expression of *uncB* in cells grown on either carbon source tended to increase during growth. Expression of *uncE*, however, was markedly higher during growth on glucose than during growth on succinate and fell during growth on either carbon source. It appears as if *E. coli* have some mechanism for specifically changing the expression of *uncE* and the resultant synthesis of the *c* subunit depending on the carbon source. Relative *c* stoichiometries were determined for F_1F_O purified from cells grown on succinate or glucose, using the same procedures described previously.

The F_1F_O purified from cells grown on glucose had significantly more *c* subunit than F_1F_O purified from cells grown on succinate (Schemidt *et al.*, 1998). Importantly, these studies showed that both the expression of *uncE* from chromosomal genes and the resultant *c* stoichiometry are variable *in vivo* with an essentially wild-type genetic background.

In a related experiment (Jensen *et al.*, 1995), an inducible *unc* operon was placed in a deletion strain and growth optima determined as a function of *unc* induction. For cells growing under aerobic conditions (with glucose or succinate), the growth optima was at a 1X level of ATPase, as measured by the amount of *c* subunit. However, under conditions of anaerobic growth on glucose, the optimal level increased to 1.4X the measured wild-type level. This again suggests that an increase of ATPase—and specifically the *c* subunit—is advantageous to the cell under anaerobic or fermentative conditions.

What are the effects on energy coupling and possible advantages to the cell of a variable *c* stoichiometry? If the *c* stoichiometry can change, then the current model of energy coupling by the ATPase would predict that the coupling ratio is also affected by these changes. It would be predicted that a change in subunit stoichiometry would have a proportional effect on the coupling ratio, *i.e.*, that as the stoichiometry increases, the coupling ratio should also increase. Not surprisingly, measurements of the coupling ratio under different growth conditions were conducted back in the heyday of bioenergetics, when issues of the coupling ratio were in vogue. In 1982 and 1983, Kashket published two papers addressing the very question of coupling ratios of cells grown in glucose/low O_2 as compared to succinate/high O_2. The results were somewhat ambiguous, depending upon the method chosen to measure $\Delta\mu_{H^+}$. They showed either no change in the coupling ratio, or perhaps a higher ratio in the glucose/low O_2 condition. Vink *et al*. (1984) also measured coupling ratios under different metabolic conditions and found that O_2 limitation increased the coupling ratio. Such an increase is what would be predicted from the experiments showing increased stoichiometry under fermentative conditions.

The stoichiometric changes determined from the carbon-source studies are opposite from what might be predicted on the basis of thermodynamic considerations. During growth on succinate, the data predict a lower coupling ratio, which would produce a lower ΔG_{ATP} for a given $\Delta\mu_{H^+}$. Conversely on glucose, the higher coupling ratio would produce a lower $\Delta\mu_{H^+}$ for a given ΔG_{ATP}. We speculate that perhaps these changes, which appear to be thermodynamically unfavorable, are kinetically favorable, producing higher rates of ATP synthesis and lower rates of ATP hydrolysis under these two different metabolic circumstances, at the expense of overall coupling efficiency. It has also been suggested that bacterial cells will sacrifice efficiency to maximize growth rate (Westerhoff *et al*., 1982).

Alternatively, the *c* stoichiometry of the synthase in the succinate-grown cells is obviously adequate for ATP synthesis and other considerations might dictate raising the stoichiometry in glucose-grown cells, *e.g.*, removal of cytoplasmic protons. It is known that cells growing on ammonium as sole nitrogen source, with a neutral carbon source (*e.g.*, glucose), have an excess of cytoplasmic protons and require either ATP or elevated respiratory rates to remove those protons (Booth, 1985). Increasing the *c* stoichiometry might be another mechanism for proton removal, so that more protons are pumped for each ATP hydrolyzed. Likewise, vacuolar proton pumps in plants show changes in subunit stoichiometry in response to salt stress (Low *et al*., 1996). Indeed, there might be no universally applicable rules to variations in *c* stoichiometry. Each organism has its own "axe to grind," so to speak, and differences in rates of proton pumping, ATPase and ATP synthase activities, and coupling ratios might all be variables in the choices that each living thing has to make to survive and compete in its own environment.

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