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The thermodynamic efficiency of ATP synthesis in oxidative phosphorylation

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HIGHLIGHTS

- The thermodynamics of the vital process of oxidative phosphorylation is investigated.
- The efficiency of ATP synthesis in OX PHOS is calculated from first principles.
- Applications in the design and fabrication of mechanochemical devices are explored.
- Some new ways to exorcise Maxwell's demon are proposed.
- Aspects of unity in biological energy transduction mechanisms are discussed.

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ABSTRACT

As the chief energy source of eukaryotic cells, it is important to determine the thermodynamic efficiency of ATP synthesis in oxidative phosphorylation (OX PHOS). Previous estimates of the thermodynamic efficiency of this vital process have ranged from Lehninger's original back-of-the-envelope calculation of 38% to the often quoted value of 55–60% in current textbooks of biochemistry, to high values of 90% from recent information theoretic considerations, and reports of realizations of close to ideal 100% efficiencies by single molecule experiments. Hence this problem has been reinvestigated from first principles. The overall thermodynamic efficiency of ATP synthesis in the mitochondrial energy transduction OX PHOS process has been found to lie between 40 and 41% from four different approaches based on a) estimation using structural and biochemical data, b) fundamental nonequilibrium thermodynamic analysis, c) novel insights arising from Nath's torsional mechanism of energy transduction and ATP synthesis, and d) the overall balance of cellular energetics. The torsional mechanism also offers an explanation for the observation of a thermodynamic efficiency approaching 100% in some experiments. Applications of the unique, molecular machine mode of functioning of F_1F_O-ATP synthase involving direct interconversion of chemical and mechanical energies in the design and fabrication of novel, man-made mechanochemical devices have been envisaged, and some new ways to exorcise Maxwell's demon have been proposed. It is hoped that analysis of the fundamental problem of energy transduction in OX PHOS from a fresh perspective will catalyze new avenues of research in this interdisciplinary field.

1. Introduction

The thermodynamic efficiency of adenosine 5′-triphosphate (ATP) synthesis in mitochondrial oxidative phosphorylation (OX PHOS) is of central importance because it constitutes the main energy source in eukaryotic cells. The ATP produced by the F_1F_O-ATP synthase is transported out of mitochondria and used for the function of nerve, muscle, liver and other tissues, for the performance of osmotic work, and for biosynthesis of the various compounds of cellular metabolism [1–4]. The synthesis of ATP is the most prevalent biochemical reaction in the human body and an active person synthesizes his own body weight of ATP every day. This fundamental mode of cellular energy production also occurs in green plants by the process of photosynthesis and in microorganisms. Yet the overall mechanistic P/O ratios and the thermodynamic efficiency of this vital process have not been definitively established and textbooks estimate the efficiency as lying between 55 and 60% [2]. They also emphasize the need to reassess these numbers as more information about “these complex coupled processes” is obtained [2]. A recent value of efficiency as high as 90% was arrived at from information theoretic considerations [3]. The purpose of this note is to show that recent developments in structural elucidation, molecular mechanism of ATP synthesis, and novel thermodynamic insights into the coupling of oxidation and ATP synthesis finally permit an exact

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estimate of the thermodynamic efficiency of oxidative phosphorylation in mitochondrial energy transduction.

2. Background

Among researchers on this subject, the present author has had the most abiding interest since the early 1990s in calculating the thermodynamic efficiencies of oxidative phosphorylation, primarily in a bid to test the predictions of a novel torsional mechanism of energy transduction and ATP synthesis [4–11]. Exact analysis was thwarted primarily by the unknown complex mechanistic details and the uncertainties in the mechanistic redox H^+/O and ATPase H^+/ATP stoichiometries (and consequently the mechanistic P/O) operative in mitochondrial OX PHOS. Additionally, the inclusion or exclusion of the additional transport proton altered the thermodynamic analysis [6,9]. For over five decades, until the *fin de siècle*, both the mechanistic H^+/O and H^+/ATP stoichiometries as also the mechanistic P/O ratios were believed to be integral values – 2 for succinate and 3 for NADH-based substrates for the mechanistic P/O – and thermodynamic calculations [4,5] were constrained to use these integral numbers; however, the continued use of integral values is a defect of recent evaluations [3]. Later, only the mechanistic H^+/O and H^+/ATP ratios were thought to be integers, with their ratio (= the mechanistic P/O) allowed to be a rational number, and again the nonequilibrium thermodynamic calculations and the overall cellular energy balance had to be revised [6]. Subsequently, in the new millennium, it was concluded by the research community that owing to the presence of conformational coupling in ATP synthase, even the mechanistic H^+/ATP need not be an integer. Since the torsional mechanism of ATP synthesis was a specific type of conformational coupling, it was not difficult to incorporate this new insight, and another round of calculations resulted [7]. Analysis was nonetheless complicated by the experimental fact that the number of c-subunits in the c-oligomer of F_1F_O was found to be variable ($n = 8$ to $n = 15$) and to depend on the organism [12–16]. It was shown that the mechanisms worked for all n, i.e., irrespective of whether symmetry existed (integral P/O) or whether there was symmetry mismatch (non-integral P/O) [7,10]; in fact, the torsional characteristics of the γ-subunit were postulated to be an intrinsic property of the enzyme that was obligatory for ATP synthesis, and the mechanism itself was proposed [17,18] before the existence of any evidence in the scientific literature for the presence of symmetry mismatch. Despite the several uncertainties, the original nonequilibrium thermodynamic approach and analysis [5] to compare various mechanisms of oxidative phosphorylation and evaluate their thermodynamic efficiencies was found useful [19–22] and has even been replicated virtually verbatim without proper reference [23,24].

3. Results and discussion

3.1. Restrictions imposed by experiment and chemical logic on possible theories and their thermodynamic consequences

Decades of experimental work on OX PHOS impose rather severe restrictions on bioenergetic theories and coupling mechanisms that can be devised, and calculations of the energetic efficiency of the coupled OX PHOS process have to be made within this restrictive framework. Laborious biochemical experiments on the operating P to O ratios in mitochondria have led to consensus values of ~2.5 for NADH-based substrates and ~1.5 for succinate as substrate [25–28]. Concomitantly, $H^+/2e^-$ stoichiometries of Complex I, Complex III, and Complex IV on the redox side in animal mitochondria have settled on values of 4, 2, and 4 respectively after much debate and considerable research work by a large number of groups [nicely summarized in ref. [2]]. On the ATP side, X-ray and cryoelectron microscopy work has converged after many fluctuations to a value of $8H^+$ for 3 ATP molecules [14,15]. This was interpreted as a mechanistic (i.e., ideal or zero loss) P/O ratio of 3.75 in mammalian mitochondria [10].

It has often been suggested by use of an additional "transport" or "neutralization" proton in the calculations that the mechanistic P/O ratio is ~2.5 [2,14]. However, if that is the case, then the above-mentioned structural information cannot be rationalized. Moreover, only the ions translocated through the access channels in the F_O portion of ATP synthase should be included in the P/O stoichiometry calculations (because these alone are competent to make ATP), and not protons transported through other transporters such as the ADP-ATP adenine nucleotide translocase (ANT) or the P_i-OH^- exchanger/carrier (PiC). In vivo, where the supply of ATP synthesized in mitochondria is coupled to the demand for ATP elsewhere in the cell (for instance by ADP and P_i as signaling molecules), the reverse ATP hydrolysis reaction ensures that no additional transport/neutralization proton is used during ATP synthesis. Hence, for all these compelling reasons, the measured consensus value of 2.50 (1.50 with succinate) is the actual, observed P/O ratio in animal mitochondria.

Finally, one has to be extremely careful in the exact definition of the overall efficiency of the energy transduction process. If over and above the stored energy captured in the pyrophosphate bonds of ATP, we consider the free energy spent in molecular recognition of protons, and include that informational energy and other energy terms in the calculation of useful work [3,16], then in principle we can approach an efficiency of 100% because of the inviolability of the principle of energy conservation. Such a definition of efficiency will not prove to be of any practical utility. Here these problems are avoided by defining the efficiency of the OX PHOS process as the maximum output work that a molecule of ATP can deliver in a user molecule (e.g. muscle actomyosin) under isothermal conditions divided by the input (redox) energy donated by a pair of electrons moving down the respiratory chain in mitochondria. The release of the ATP molecule's stored electrostatic energy (specifically by reducing the binding of inorganic phosphate to the enzyme after cleavage of the terminal γ-phosphorus—oxygen bond in the process of ATP hydrolysis, thereby allowing the P_i to move away from the bound $MgADP^-$, and finally ejecting it into the medium, i.e. to infinity) and its subsequent utilization in the user molecule can indeed occur with an efficiency that approaches 100%, as discussed [8,29,30] because it is a case of direct conversion of electrical energy to mechanical energy (specifically storage of the change in electrostatic potential energy upon ATP binding, hydrolysis and P_i release as torsional energy within a local domain of the macromolecule according to the unified theory, followed by release of the torsional energy along specific mechanical degrees of freedom to perform useful external work) [7,8]. However the statement that energy transduction occurs with an efficiency approaching 100% [3,16] cannot be generalized to all natural processes. Thus, the uphill transport processes on the redox side will incur substantial energy losses as these are *active transport* processes that work against the concentration gradient; such chemical pumping steps are therefore not expected to occur at close to 100% efficiency as postulated. All the above aspects need to be considered in a true theory of molecular energy transduction.

3.2. Estimation of OX PHOS thermodynamic efficiencies from consensus values arrived at by structural and biochemical experiments

As discussed recently [10], the ideal, mechanistic P/O ratio in animal mitochondria based on structural information on OX PHOS Complexes I-V for NADH-based substrates that utilize all three redox coupling sites works out to be $10/(8/3) = 15/4 = 3.75$. The actual operating P/O ratio under these conditions is the consensus experimental value of 2.50. Hence the thermodynamic efficiency of mitochondrial OX PHOS on the redox side, $\eta_{redox} = (2.50 / 3.75) \times 100 = 66.66\%$. This value could also have been derived from the excellent biochemical measurements of the affinities of phosphorylation and oxidation [25–27] (A_P and A_O, which are the quantities conventionally used in the literature to denote the thermodynamic driving forces of phosphorylation and respiration, and are equal in magnitude but opposite in sign to

ΔG_P and ΔG_O, the free energy change of ATP synthesis and respiration respectively – also sometimes called the phosphorylation potential and redox potential respectively). The value of the affinity ratio, $x = A_P / A_O$ of −0.2666 (the negative sign implies that the two processes are opposite in the sense of ion translocation, and that one process releases free energy while the other utilizes it) was extensively used in previous thermodynamic analyses [5,6], where x represents the fraction of the OX PHOS system's redox energy to synthesize one ATP molecule [4]. Hence the maximum number of ATP molecules that can be synthesized by a pair of electrons from NADH to O_2 equals $1/x = 1/0.2666 = 3.75$, and this number is therefore the ideal, zero loss mechanistic P to O ratio. This ideal value resulted from thermodynamic force (potential) measurements. The thermodynamic flux measurements led to the consensus P per $2e^-$ of 2.50 as discussed above, which is the actual, observed value of the P to O ratio. Hence once again, the biochemical measurements of thermodynamic flow and thermodynamic force were pointing to a value of $\eta_{redox} = (2.50 / 3.75) \times 100 = 66.66\%$. In summary, both structural and biochemical measurements are now seen to converge to the same value of efficiency of 66.66% on the redox side of the OX PHOS process for NADH-based substrates.

The value of efficiency on the ATP side, η_{ATP} can also be estimated from such experimental data. Since A_O for NADH-based oxidation substrates measures 220 kJ/mol (or 2280 meV, corresponding to a standard reduction potential of 1.14 V) [1,2], the phosphorylation $\Delta G_P = A_O x = 220 / 3.75 = 58.66$ kJ/mol. However, in a molecular machine involving nonequilibrium conformational states, the energy transduction is primarily governed by the ΔH part of ΔG, and this enthalpy or internal energy is stored in a submolecular element of a single F_1F_O ATP synthase molecule and is subsequently utilized to synthesize ATP; in other words, the potential energy stored in the single molecule is a consequence of a microscopic process in the membrane-bound F_O [1,4]. This is in stark contrast to Mitchell's designation of the energy transduction process as a fuel cell [31], where the accumulation of potential energy in a bulk aqueous phase is treated as a macroscopic process, and the transfer of $2e^-$ does not cause any significant increase in $\Delta\mu_H$, and hence does not lead to ATP synthesis, unlike in the case of energy transduction based on the paradigm of a molecular machine. When the mechanism is molecular, only the standard state phosphorylation potential $\Delta G_P^{0'}$ is competent to be stored in the ATP molecule – the ΔS part of ΔG cannot be stored in a single molecule. This value of $\Delta G_P^{0'}$ under real biological operating conditions of 37 °C, pH 7.5, I 0.2, and 1 mM Mg^{2+} was found to be 36 kJ/mol based on computer-aided thermodynamic calculations involving multiple ionizations by the classical work of Guynn and Veech [32] and by us based on a general electrostatic theory for energy transfer from ATP [8]. A $\Delta G_P^{0'}$ of 36 kJ/mol was also calculated by a pioneering Legendre-transformed Gibbs free energy approach for biochemical reactions involving ATP synthesis [33,34]. A similar but slightly lower value of ~35 kJ/mol for $\Delta G_P^{0'}$ under these conditions was obtained by Slater and colleague based on evaluation of the equilibrium constant for the glutamine synthetase reaction [35]. From all the above information, the thermodynamic efficiency on the ATP side, η_{ATP} works out to be $(36 / 58.66) \times 100 = 61.37\%$.

Hence the overall thermodynamic efficiency of the OX PHOS process in animal mitochondria, $\eta = (\eta_{redox} \times \eta_{ATP})/100 = (66.66 \times 61.37) \times 100 = 40.91\%$ or ~41%.

3.3. Calculation of OX PHOS efficiencies based on nonequilibrium thermodynamics

The value of η_{redox} can also be computed from first principles based on a nonequilibrium thermodynamic analysis and details will not be repeated here. The result of Fig. 2 of ref. [5] shows that a redox efficiency of ~66% is obtained at a $\Delta G_P/\Delta G_O$ or affinity ratio of −0.2666 for oxidative phosphorylation by rat liver mitochondria for NADH-based oxidation substrates. On the ATP side the value of η_{ATP} is given by $(\Delta G_P^{0'} / \Delta G_P) \times 100$, which yields a value of $(36 / 58.66) \times 100 = 61.37\%$. Hence the overall thermodynamic efficiency of OX PHOS, η based on nonequilibrium thermodynamics equals approximately 40–41%.

3.4. Interpretation and calculation of thermodynamic efficiencies of OX PHOS based on molecular mechanistic considerations

As discussed above, the affinity of oxidation for sites 1 + 2 + 3 per $2e^-$ from NADH to oxygen is 220 kJ/mol, while the phosphorylation affinity in mitochondria works out to be −58.66 kJ/mol. A magnitude of ΔG_P lower than 58.66 kJ/mol is insufficient to synthesize an ATP molecule in mitochondrial OX PHOS in state 3. In other words, a mitochondrial F_1F_O-ATP synthase enzyme molecule (MF_1F_O) must provide at least 58.66 kJ/mol per ATP in order that the activation energy barrier is overcome and an ATP molecule is synthesized during operation in state 3. This value of 58.66 kJ/mol is interpreted as the quantum of torsional energy stored in a single MF_1F_O molecule, first in the 8 c-subunits of the c-oligomer as twist, and subsequently ~54 kJ/mol in the central γ-subunit as torsion. Of this, 4.66 kJ/mol twist energy is lost to set the MF_O system into rotation from its local equilibrium state. Another ~18 kJ/mol of torsional energy is used to strain the ε-β_E bond so that the sum of MgADP binding energy in β_E (~27 kJ/mol) and the torsional energy (~18 kJ/mol) can just overcome the ε-β_E interaction energy, which in turn had just exceeded the binding energy of MgATP in the tight site ($\beta_{DP\text{-}like}$) of ~44 kJ/mol and had converted the tight site to the open, distorted β_E site via interaction with the rotating ε-subunit and thereby help release product ATP in the previous one-third of the catalytic cycle in the F_1 portion of ATP synthase. The balance γ-subunit torsional energy of ~36 kJ/mol is used to force HPO_4^{2-} from infinity to a bonding distance from $MgADP^-$ of 0.3 nm in $MgATP^{2-}$, and this ~36 kJ/mol is stored electrostatically in $MgATP^{2-}$ (with respect to the reactant $MgADP^-$ and HPO_4^{2-} species when they are located at an infinite separation distance from each other). In the process of ATP hydrolysis when the terminal γ-phosphorus—oxygen bond is cleaved, and the binding affinity of inorganic phosphate to the enzyme is progressively reduced, the HPO_4^{2-} moves away from bound $MgADP^-$ and is finally ejected into the external medium, i.e. to infinity, and the standard free energy change of ATP ($\Delta G_P^{0'}$) of ~36 kJ/mol is available for performance of useful mechanical work in the user molecule [4,7,8].

The question arises why nature devised two types of energy-transducing systems in mitochondrial respiration, i.e. Complex I and Complex IV which pump $4H^+$ per $2e^-$ and Complex III which has half the stoichiometry of $2H^+$ per $2e^-$ [or in photosynthesis why did nature evolve two types of Photosystems, i.e. PS I (with a stoichiometry of $2H^+/2e^-$) and PS II (with a stoichiometry of $4H^+/2e^-$)]? The answer cannot be found at the level of protons, which is the same species transported by both types of transporters, but lies in the *coupling* of proton and anion transport in response to electron transfer within these enzyme complexes. This has the important thermodynamic consequence that the energy-transducing complexes of OX PHOS and photosynthesis are proton-dicarboxylic acid cotransporters and not simply electrogenic proton pumps as in the chemiosmotic theory, and that in mitochondria the protons do not originate from water as in chemiosmosis, but rather from succinic acid [4,9–11,36–38]. Thus, Complexes I and IV in mitochondria (and PS II in green plants) are specific to recognition, binding and translocation of dicarboxylic acid dianions and protons, while Complexes II-III (and PS I in green plants) specifically bind and translocate only the dicarboxylic acid monoanion and H^+ in response to e^- transfer. The H^+ stoichiometry of Complex I and IV is twice that of Complex III because twice the number of protons are required to neutralize the succinate dianion compared to the succinate monoanion. In mitochondria, Complex V (F_1F_O-ATP synthase) is permeant to the succinate monoanion and H^+, but impermeant to the succinate dianion. Translocation of H^+ and succinate monoanion (and in some organisms, a countercation such as Na^+ or K^+) each contribute ~50% of the total energy required to synthesize ATP. The succinate monoanion and H^+ translocate through their respective access

channels in a- and c-subunits in an ordered and sequential way, so that a local $\Delta\psi$ [4,9–11,39–41] is created in F_O transiently between the monoanion and proton translocations. This local potential is destroyed by the secondary translocation and the change in potential is transduced by a cascade of events to torsional energy in the γ-subunit and finally into stored energy of ATP in a catalytic cycle by means of a torsional mechanism [4,6–11,17,18].

At a pH of ~5.0 in the intracristal space (which is different in ionic composition, pH, and distribution of carrier proteins from the intermembrane space in mitochondria) [42–44], only ~70% of the H^+ are in H^+A^- form (Fig. 1) and are competent to make ATP. Thus, of 10 protons and anions translocated per $2e^-$ into the intracristal space, only 7 are useful in ATP synthesis through Complex V, while the neutral (H_2A) and dianion ($2H^+$ A^{2-}) forms constitute redox losses as leak and slip respectively. Hence a novel *molecular* explanation is provided for the first time to macroscopic leak and slip phenomena that have been known to exist since a long time in biological membranes involved in mitochondrial bioenergetics [45–47]. In the pH ~7.5–8.0 matrix compartment all the anions exist as the succinate dianion; hence translocation of A^{2-} and $2H^+$ is a downhill event from pH ~8 (matrix space) to pH ~5 (intracristal space) that entails negligible losses. However, per $2e^-$, the 2 A^-H^+ are pumped uphill by Complex II–III against a concentration gradient initially of 1:7.

Hence per $2e^-$, the free energy losses (ΔG_1) for the first translocation,

$$\Delta G_1 = \frac{RT}{1000} \ln \frac{C_{out}}{C_{in.}}$$

$$= (8.314 \times 310/1000) \times \ln 7 = 5.02 \text{ kJ/mol}$$

For the second translocation we have to work against an ~eightfold gradient (due to the initial + first translocation above), i.e.

$$\Delta G_2 = \frac{RT}{1000} \ln \frac{C_{out}}{C_{in}}$$

$$= (8.314 \times 310/1000) \times \ln 8 = 5.360 \text{ kJ/mol}$$

Therefore, active transport losses per $2e^- = 5.02 + 5.36 = 10.38$ kJ/mol.

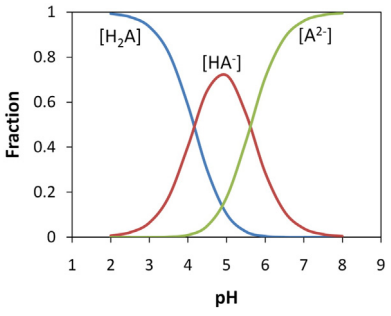

Fig. 1. Distribution of [HA^-], [H_2A] and [A^{2-}] forms of succinic acid as a function of pH. Molecular interpretation in mitochondrial energy transduction in state 3 (see text also): [HA^-] is the motive monoanionic form competent to synthesize ATP on symsequenceport with protons through the membrane-bound F_O portion of the F_1F_O-ATP synthase, which functions as a proton-dicarboxylic acid anion cotransporter; [H_2A] is the neutral permeant form ("leak") that diffuses through the membrane across the a-c lipid-water interface in F_O and is uncoupled to ATP synthesis; [A^{2-}] is the dianionic form of the succinic acid pumped out by the redox Complexes I and IV ("slip") but is impermeant to the mitochondrial MF_1F_O-ATP synthase (Complex V). Note that in such a dicarboxylic acid system, there is no pH at which 100% [HA^-] species can be generated, i.e., the reaction of H_2A to HA^- can be said to be complete while the conversion of HA^- to A^{2-} in a second step has occurred to only a negligible extent. In fact, analysis of ionization behavior and inspection of the species distribution curves above show that the fraction of [HA^-] can never exceed ~0.73 for the succinic acid system.

The redox free energy available after accounting for pumping losses by the respiratory chain for the NADH–NAD^+ couple = 220–10.38 = 209.62 kJ/mol. However, 30% of the redox energy is lost as leak and slip and only the fraction 7 / (1 + 2 + 7) = 0.70 is useful for making ATP; hence the redox free energy available after accounting for losses due to leak and slip = 209.62 × 0.70 = 146.70 kJ/mol.

Thus, the thermodynamic efficiency on the redox side, $\eta_{|redox}$ in animal mitochondria from the molecular mechanism = (146.70 / 220) × 100 = 66.68%. The self-consistency of such mechanisms should be stressed because 146.70 / 58.66 = 2.50, which is a mechanistically derived value of the operating P/O ratio, and 220 / 58.66 = 3.75, which is the ideal P/O ratio. Hence a value of $\eta_{|redox}$ of (2.50 / 3.75) × 100 = 66.66% is rationalized from the mechanistic analysis also. The thermodynamic efficiency on the ATP side, $\eta_{|ATP}$ = (36 / 58.66) × 100 = 61.37% because the appropriate parameter for analysis of energy transduction by an enthalpy-driven molecular machine is the standard free energy change under the actual operating conditions of T, pH, I, and pMg, but not the complete thermodynamic function (with the concentration-dependent term). Hence the overall thermodynamic efficiency of OX PHOS, η of mitochondria operating in state 3 from mechanistic considerations based on the torsional mechanism = ($\eta_{|redox} \times \eta_{|ATP}$) / 100 = 40.92% or ~41%.

3.5. Calculation of an overall OX PHOS efficiency based on the cellular energetics balance

The complete oxidation of a glucose molecule results in the formation of a lower limit of 32 molecules of ATP [1–3]. Taking the estimated standard Gibbs free energy change of glucose oxidation value of 2808.96 kJ/mol [6], we have as the input $\Delta G_O^{0'}$ per glucose approximately 2809 kJ/mol. The output per glucose molecule yields a standard Gibbs free energy store of 32 × 36 kJ/mol = 1152 kJ/mol. Hence the overall η measures (1152 / 2809) × 100 = 41.01%. If a value of standard Gibbs free energy change of 2867.5 kJ/mol per glucose molecule [1,2] is used, then the overall η is 40.17%. Hence an overall η based on the balance of cellular energetics works out to be ~40–41%.

4. Summary and conclusions

The overall thermodynamic efficiency of ATP synthesis in the vital process of oxidative phosphorylation in mitochondria has been shown to be 40–41%. An identical value of efficiency has been shown to result from four different approaches based on a) estimation using structural and biochemical data, b) fundamental nonequilibrium thermodynamic analysis, c) novel insights arising from Nath's torsional mechanism of energy transduction and ATP synthesis, and d) the overall balance of cellular energetics. Previous estimations of the thermodynamic efficiencies varied considerably from Lehninger's original back-of-the-envelope calculation of 38% [48], to the often quoted value of ~60% by current textbooks [2], to high values of ~90% by a recent information theory approach [3], and even realizations of close to ideal 100% efficiencies [29,30]. The convergence of efficiency calculations by four independent approaches a) to d) above, each with a sound theoretical basis, lend great confidence that the calculated η of ~40% is the definitive value of the thermodynamic efficiency of ATP synthesis in the OX PHOS process. Textbooks should consider incorporating this value in future editions.

A merit of this analysis lies in the fact that the definition of thermodynamic efficiency is based on the ratio of the final output Gibbs free energy (energy stored in the ATP molecule and therefore available for performance of external work in a user device) to the total initial input Gibbs free energy (the redox energy of the NADH–NAD^+ couple per $2e^-$). Hence all molecular recognition and "information gathering" [3] events have already been subsumed in the analysis, and all losses on the redox side and on the ATP side have also been accounted for in the calculations.

The molecular mechanism of e^--coupled H^+ translocation by Complexes I–IV on the redox side in mitochondria has remained unsolved despite major efforts; nonetheless, a thermodynamic η_{redox} could still be calculated, thanks to novel insights into the ionization properties of the two concentration gradients coupling respiration and phosphorylation. On the ATPase side, analysis was greatly facilitated by the availability of a detailed Nath's torsional mechanism of ATP synthesis [4,7] and ATP hydrolysis [7,8]. In chloroplasts, a very similar energy transduction mechanism operates with the intracristal space of mitochondria replaced by the chloroplast intrathylakoid lumenal space (pH = 5.0), the mitochondrial matrix space by the chloroplast stromal space (pH = 8.0), and with malic acid as the dicarboxylic acid involved in the photophosphorylation and photosynthesis process [10,11] instead of succinic acid in OX PHOS (Fig. 1). With the help of these novel insights, it can be truly said that the fundamental mechanisms of biological energy transduction reveal bioenergetic aspects of unity at the biochemical and physiological levels.

The work also offers a delineation of the situations in which the thermodynamic efficiency can approach 100% and a convincing explanation of the underlying reasons. Such high efficiency energy conversion has been readily shown to occur during the direct inter-conversion of chemical and mechanical energy, when an energy source of *high quality* (i.e. an energy source characterized by a low value of $\Delta S/\Delta U$ or $\Delta S/\Delta H$) transduces the stored energy into useful work without equilibration with the thermal degrees of freedom of the surrounding medium. Thus in muscle, the S-2 coiled coil of a single myosin molecule is common to two heads, and each head can bind to a different actin filament, and hence the two heads can execute two simultaneous power strokes (on different actin filaments) per ATP using the stored torsional energy of ~36 kJ/mol in the S-2 coiled coil by a rotation-uncoiling-tilt energy storage mechanism of muscle contraction [7,8]. This leads to a work output of 2×5.7 pN $\times$ 5.3 nm = 60 zJ per ATP, or ~36 kJ/mol. Similarly, during ATP hydrolysis by F_1-ATPase, a MgATP binding energy of ~36 kJ/mol in the site with intermediate affinity (site 2 or β_{TP}) and an energy of ~18 kJ/mol available upon P_i release leads to a rotational work output of the γ-subunit of ~42 pN.nm $\times$ $2\pi/3$ = 90 zJ [30], which corresponds to an energy of (~36 + ~18) or ~54 kJ/mol, an efficiency of energy conversion of approximately 100%.

Biology has gained enormously from advances in physics, chemistry, and engineering during the past hundred years. All instruments, measurement techniques, and theories directly borrowed from the physical and chemical sciences have greatly enhanced biological research. But it begs the question, as to what biology has to offer these sciences? Biologically-inspired molecular machines with their unique mode of functioning could prove to be an important contribution to physics, chemistry and engineering [6,49]. Man could not conceive of such molecular machines, and the machines designed by man do not function by this unique, high-efficiency pathway of a work-to-work conversion without thermal intermediates. Thus, the *direct* inter-conversion of chemical and mechanical energy is not an element of our industrial design yet, although it is the most ubiquitous molecular energy transduction occurring in biology, in the F_1F_O-ATP synthase, in muscle, and in other related biological systems. This offers great hope and opportunity for the design and fabrication of novel mechanochemical machines and devices in the future.

Finally, a recent work invokes the F_1F_O-ATP synthase enzyme's action as a Maxwell's demon to explain its high efficiency of energy conversion [3]. However, by considering the relevant *nonequilibrium* states with their intrinsic dynamical timescales and differing lifetimes between the initial and final equilibrium states of the molecular motor, and by excitation of specific mechanical degrees of freedom that do not exchange with the thermal degrees of freedom of the surroundings within the characteristic time of operation of the motor, and a store of the required quantum of energy in its enthalpic form in the various nonequilibrium conformational states of the machine [50], problems with Maxwell's demon are avoided (see especially pp. 1812–1815 in ref. [7]). Such an exorcisation of Maxwell's demon is different from those attempted by other workers though details are beyond the scope of this work. In brief, Szilard [51], and later Brillouin [52] emphasized the thermodynamic cost of the measurement step; using light as the measuring agent, Brillouin [52] showed that the increase in entropy of the system due to this step offsets the decrease in entropy that the demon can effect. However, Bennett and co-workers proposed that the measurement step need not be thermodynamically costly; they attributed the increase in entropy of the system to the final resetting step of the engine [53] rather than to the measurement step per se. The alternative solution eliminates a major role for entropy changes in the functioning of the molecular machine and focuses primarily on the enthalpic nature of the stored energy in the device. This does not imply that there are no losses in the operation of such a nonequilibrium engine. However, now the losses are not incurred during the measurement step or during the final resetting step, but in the initial step that converts a local equilibrium state into a nonequilibrium state that allows motion of the engine and its transition back to the local equilibrium state [4]. Hence, paradoxically, an in-depth analysis of the molecular machines in the biological world should also help provide new insights into the complex physicochemical problems associated with a hypothetical being invoked by Maxwell, problems that have intrigued scientists now for more than a century.

It is hoped that analysis of the fundamental problem of mitochondrial energy transduction from a fresh perspective will stimulate students, teachers and researchers alike, and catalyze new avenues of research in the field.

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