Energy Transfer from Adenosine Triphosphate: Quantitative Analysis and Mechanistic Insights

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The ATP−ADP thermodynamic cycle is the fundamental mode of energy exchange in oxidative phosphorylation, photophosphorylation, muscle contraction, and intracellular transport by various molecular motors and is therefore of vital importance in biological energy transduction and storage. Following a recent suggestion in the pages of this journal (Ross, J. *J. Phys. Chem. B 2006, 110*, 6987−6990), we have carried out a simple quantitative analysis of a direct molecular mechanism of energy transfer from adenosine triphosphate (ATP). The simulation provides new insights into the mechanistic events following terminal phosphorus−oxygen bond cleavage during ATP hydrolysis. This approach also allows for the division of the energy-transfer process into elementary steps and for the prediction of the distribution of the standard-state Gibbs free energy of the overall ATP hydrolysis process among the various steps of substrate binding, bond cleavage, and product release in the enzymatic cycle, which had proved very difficult to specify previously. These predictions are consistent with available experimental data on ATP hydrolysis by protein biomolecular machines. The fundamental biological implications arising from our results are also discussed in detail. The aspects considered in this work enable us to look at the entire process of ATP synthesis/hydrolysis and energy transduction and storage in various biological molecular machines in a logically consistent and unified way.

I. Introduction

Understanding the process of oxidative phosphorylation and the fundamentals of the mechanism of the synthesis of adenosine triphosphate (ATP), the universal biological energy carrier, and its hydrolysis by various molecular machines of the mammalian cell has stimulated considerable research work over the past few decades.[1−10] The free energy liberated by the hydrolysis of ATP to ADP and inorganic phosphate is utilized to drive reactions requiring an input of free energy, such as muscle contraction.[10−15] Because green plants and microorganisms also capture and utilize energy by the same reaction,[16−18] the ATP−ADP thermodynamic cycle is the universal mode of energy exchange in biological systems. This is therefore a very important type of energy transduction in all of biology, and we are challenged to understand the molecular mechanism of this vital process.

Mechanisms of energy transduction into/from ATP can be divided into two classes: direct and indirect. A detailed molecular mechanism of *direct* transduction of chemical energy into mechanical energy and vice versa that has been proposed is the torsional mechanism of energy transduction and ATP synthesis/hydrolysis.[9,10,15,19−27] In this molecular mechanism, the crucial role of electrostatic interactions between two charges (such as MgADP and P_i) in order to effect dissipation-free energy transduction was emphasized.[10,21,27] The ATP molecule itself was modeled as two negatively charged spheres attached to the ends of two hinged bars by an inextensible string forcing the spheres to remain close to each other in a high-energy conformation. ATP hydrolysis was considered analogous to cutting the string, thereby freeing the charged spheres and allowing them to move away as a result of mutual electrostatic repulsion. This movement of charges was conceived to carry out useful work such as rotation of the γ-subunit in F_1-ATPase or to be transduced and stored as in the uncoiling of the S-2 coiled coil in myosin during muscle contraction.[10,14,15,27] Recently, a suggestion along these lines was also made in the literature by Ross.[28] In this interesting suggestion, Ross envisages that the entire standard-state free energy of ATP hydrolysis, $\Delta G^{0\prime}$, is released not during the bond cleavage step but subsequently, after the binding of the charges to their sites is reduced.[28] This suggestion helped us to formulate an explanation of the mechanism of hydrolysis of ATP in which the release of the energy transduced at the cleavage step was distributed over other elementary steps of the enzymatic cycle, especially during the release of the inorganic phosphate into the medium.[27] Here, we develop this insight mechanistically as well as quantitatively and show the interesting results of a simulation that help to further elucidate the molecular mechanism of ATP hydrolysis and energy transfer.

II. Current Views of Energy Transduction by ATP-Utilizing Biological Molecular Machines

Various views of energy transduction have been put forth in the scientific literature. According to several researchers in bioenergetics, the actual chemical synthesis of ATP (and thus cleavage of ATP in the hydrolysis mode) does not require any external energy input. They propose that the release of bound

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ligands (e.g., MgADP, P_i) is the major energy-requiring step and that this energy is provided by MgATP binding and transmitted from the substrate catalytic binding site to the product release catalytic site.[6,7,29] However, such a long-range transmission of energy has not been verified by researchers despite the passage of almost four decades, which is a major drawback. On the other hand, many researchers in the vast field of motility make the assumption that the release of bound ligands drives muscle contraction/performs useful work and that force production is directly coupled to these release steps, which is quite opposite to the viewpoint of certain researchers in bioenergetics. Workers in both of these fields are persuaded that there is considerable experimental evidence in favor of their respective views and are therefore reluctant to alter their stands.

Various attempts have been made to resolve the above long-standing difficulties in these fields. Notable among these are the proposals of the torsional mechanism of energy transduction and ATP synthesis and hydrolysis,[9,10,15,19−27] the rotation−uncoiling−tilt (RUT) energy-storage mechanism of muscle contraction,[10,14,15,26,27] the rotation−twist (RT) energy-storage mechanism for processive molecular machines,[15,26,27] and the recent suggestion made by Ross.[28] According to the torsional mechanism of ATP synthesis, every elementary step requires energy,[9,10,20−22,24,27] including chemical synthesis, and hence, cleavage and Coulombic repulsion between the charged cleavage products MgADP and P_i releases free energy, contradicting a central tenet of the binding change mechanism (in which $\Delta G^{0\prime}$ for this step was assumed to be zero).[6] Recently, Ross made the valuable suggestion that the energy of the Coulombic repulsion between the charged products could be harnessed for useful work in subsequent elementary steps where the binding of the product was reduced.[28] This suggestion has been incorporated in a unified theory of ATP synthesis/hydrolysis and the thermodynamics of the ATP−ADP couple.[27] These fundamental advances allowed us to explain all of the apparently disparate experimental observations in bioenergetics and motility and to satisfy both poles of viewpoints mentioned above. However, now, the basic tenets of the binding change mechanism such as the energetically free nature of the actual chemical synthesis/cleavage elementary step, the free rotation of the γ-subunit in F_1-ATPase, and the transmission of binding energy from one catalytic site to another distant catalytic site had to be abandoned and replaced by the corresponding postulates of the torsional mechanism (e.g., storage of torsional energy in the γ-subunit in F_1F_O-ATP synthase that is subsequently utilized to cause conformational changes in the β-catalytic sites that lead to ATP synthesis).[9,10,27]

III. Further Development and Analysis of Recent Suggestions on Energy Transduction in the ATP Hydrolysis Mode

Here, we have further rationalized and developed the suggestions in our previous work[10,27] and the recent work of Ross[28] and have interpreted the results in a molecular mechanistic setting where ATP hydrolysis and the electrostatic repulsion between charges occur in various biomolecular motors. The exact distribution of free energy between the terminal γ-phosphorus−oxygen bond cleavage step and the product release elementary steps could not be specified earlier, owing to the qualitative nature of this aspect of the proposals.[10,28] Such quantification of the energy distribution of the fundamental ATP−ADP couple has also not proved to be easy to determine for F_1-ATPase or conventional muscle myosin because of various technical difficulties, the fast nature of the reactions, and the very nature of the power strokes. For instance, in muscle mechanics, as per the tenets of the RUT mechanism, the excess binding energy of MgATP binding (i.e., the free energy over and above that required to unbind myosin head from actin), part of the free energy of ATP hydrolysis, and the stored Coulombic repulsion energy between the cleaved products (available upon release of P_i to infinity) are accumulated and stored in a localized region of myosin II (specifically in an uncoiled high-free-energy state of the S-2 coiled coil) and are released together in a single step and used to cause *simultaneous* power strokes by both myosin heads of the double-headed myosin molecule.[10,14,15,27] However, an analysis of the motility of cargo-transporting double-headed processive motors on microtubule or actin tracks can help solve this problem because, in these cases of intracellular transport machinery, each head of the double-headed motor steps forward *alternately* rather than simultaneously.

As discussed above, the processive motility of several classes of double-headed molecular motors is characterized by *sequential* movement of each of the heads.[15,27,30−33] Because the step size of forward movement by each head is the same and each head moves against the same backward load in various single-molecule optical trapping experiments, we deduce that the two heads of the motor utilize the same quantum of free energy for forward processive movement. Further, the movement of one head takes place before P_i release occurs, whereas the other head moves forward only after P_i is released as a result of interactions of the microtubule/actin track with the motor head.[15,26,27,30,31] This leads us to conclude that the total standard-state free energy of ATP hydrolysis is distributed equally between events before and after P_i release. These insights permit us to formulate a quantitative electrostatic model.

IV. Electrostatic Model of Energy Transfer in ATP

The change in electrostatic potential energy between the bound charges $MgADP^-$ (z_A) and HPO_4^{2-} (z_B) at an initial separation r_i and a final separation r_f is given by the basic expression

$$\Delta E = \frac{z_A z_B e^2}{4\pi\varepsilon_0\varepsilon}\left(\frac{1}{r_i} - \frac{1}{r_f}\right) \quad (1)$$

where e is the electronic charge, ε_0 is the permittivity of vacuum, and ε is the dielectric constant of the medium. The initial P—P distance in ATP is r_{init}, which increases upon terminal P—O bond cleavage to 0.4 nm, which is twice the ADPO—P distance in the transition state.[8] The electrostatic energy released depends on the exact value of ε in the catalytic binding site where the hydrolysis event occurs, which, however, is unknown. We can say that the P—P distance of r_{init} corresponds to that in tightly bound MgATP, whereas the distance of 0.4 nm corresponds to the situation in the MgADP·P_i state immediately upon bond cleavage where the products are tightly bound in the catalytic site. As suggested before, unless the binding of inorganic phosphate to the catalytic site is weakened, the P_i cannot move away from the MgADP, and hence, the Coulombic repulsion energy cannot be released.[27,28] However, a source of energy is needed to weaken P_i binding to its catalytic site, and we suggest here that the energy released, ΔE_1, upon increase of the distance between the charges from r_{init} to 0.4 nm as a result of the bond cleavage event is utilized to weaken the binding of P_i to its site. This weakening of P_i binding allows it to move farther away from the MgADP, and a local equilibrium is attained when

the distance between the charges is r. This movement results in a release of electrostatic energy, ΔE_2. In this state, P_i is completely unbound or very loosely bound to its catalytic site. Hence, a small conformational change resulting from the interaction of another subunit (e.g., γ-subunit in F_1-ATPase) with the catalytic site is sufficient to release P_i into the external medium (i.e., to infinity from MgADP). This increase in distance between the charges from r to infinity results in a further release of electrostatic potential energy, ΔE_3. Because the change in energy upon P_i release to the medium equals the sum of energy released in the steps prior to P_i release, as interpreted from data on double-headed processive motors discussed in section III, we can write the equation

$$\Delta E_1 + \Delta E_2 = \Delta E_3 \tag{2}$$

It should be noted that eq 2 should hold true for all biomolecular machines utilizing the ATP−ADP cycle, although it is not possible to derive this equation for F-type ATPase or myosin motors directly. This is because of the expected universality of the thermodynamics of the ATP−ADP cycle in biological molecular machines, as the same process with the same sequence of elementary steps takes place in all such machines. The magnitudes of ΔE_1, ΔE_2, and ΔE_3 can differ among different machines depending on the local conditions prevailing at the site. Moreover, the sum of ΔE_1, ΔE_2, and ΔE_3 must equal the standard-state Gibbs free energy change

$$\Delta E_1 + \Delta E_2 + \Delta E_3 = \Delta G^{0\prime} \tag{3}$$

Because the value of $\Delta G^{0\prime}$ is a function of pH, ionic strength, pMg, and temperature, the estimates of the magnitudes of ΔE_1, ΔE_2, and ΔE_3 can further vary depending on the experimental conditions.

Application of eqs 1 and 2 leads to

$$\left(\frac{1}{r_{\text{init}}} - \frac{1}{r}\right) = \frac{1}{r} \tag{4}$$

from which the intermediate distance between the charges, r, works out to be $2r_{\text{init}}$. This estimate is independent of the value of the medium dielectric constant.

For a particular value of the P—O bond distance and a selected value of $\Delta G^{0\prime}$, it is possible to evaluate the unknown local dielectric constant using the calculated value of r from eq 4, and an iterative computer program that simulates eqs 1−3 was written to carry out these calculations. P—O bond lengths between 0.145 and 0.155 nm were selected (in steps of 1×10^{-3} nm), and the value of $\Delta G^{0\prime}$ was varied between 30 and 40 kJ mol^{-1} (in steps of 1 kJ mol^{-1}) to encompass the entire range of thermodynamic standard-state free energy data under various experimental conditions. The program output included ΔE_1, ΔE_2, ΔE_3, and ε. A unique solution that satisfied eqs 1−3 was found under all conditions. The solution obtained was also verified by substitution into eq 1.

V. Results and Discussion

Selected results from the computer output are given in Table 1a−c. In the ideal case, we expect the values of ΔE_1 and ΔE_2 to be equal, based on mechanistic considerations. This is because the standard-state Gibbs free energy released upon terminal P—O bond cleavage (which is employed for the reduction of P_i binding to its binding site) will cause P_i to move away from MgADP to the intermediate distance r for which the reduction of Coulombic repulsion energy by this outward movement equals the reduction of binding energy of P_i to its site. Because the forces involved are all conservative, we expect no losses of free energy in the ideal situation. In any case, the reduction in binding energy by a certain amount will not allow the reduction in electrostatic energy by a greater amount because of energy balance considerations, as then there would be no explanation for the source of the extra energy required for the reduction of Coulombic repulsion. Hence, we can eliminate all of the entries in Table 1b, for which $\Delta E_2 > \Delta E_1$. For the values in Table 1c, $\Delta E_1 > \Delta E_2$, which is a possibility, but one indicating dissipative losses. For the most efficient machine, we expect these losses to be negligible and, hence, ΔE_1 to be equal to ΔE_2. Further, P—O bond lengths less than 0.150 nm are unlikely based on bond length data. Hence, we need not consider the entries in Table 1c. Therefore, we are left with the entries in Table 1a, which show $\Delta E_1 = \Delta E_2$ for all values of $\Delta G^{0\prime}$.

TABLE 1: Computed Values of Potential Energies and Dielectric Constants for Various Values of $\Delta G^{0\prime}$ for P—O Bond Lengths of (a) 1.50, (b) 1.55, and (c) 1.45 Å for the Electrostatic Model of Energy Transfer in ATP

$\Delta G^{0\prime}$ (kJ mol^{-1})	ε	ΔE_1 (kJ mol^{-1})	ΔE_2 (kJ mol^{-1})	ΔE_3 (kJ mol^{-1})
		(a) P—O = 1.50 Å		
30	30.84	7.5	7.5	15
31	29.84	7.75	7.75	15.5
32	28.91	8	8	16
33	28.03	8.25	8.25	16.5
34	27.21	8.5	8.5	17
35	26.43	8.75	8.75	17.5
36	25.70	9	9	18
37	25.00	9.25	9.25	18.5
38	24.35	9.5	9.5	19
39	23.72	9.75	9.75	19.5
40	23.13	10	10	20
		(b) P—O = 1.55 Å		
30	29.84	6.75	8.25	15
31	28.88	6.975	8.525	15.5
32	27.98	7.2	8.8	16
33	27.13	7.425	9.075	16.5
34	26.33	7.65	9.35	17
35	25.58	7.875	9.625	17.5
36	24.87	8.1	9.9	18
37	24.20	8.325	10.175	18.5
38	23.56	8.55	10.45	19
39	22.96	8.775	10.725	19.5
40	22.38	9	11	20
		(c) P—O = 1.45 Å		
30	31.90	8.25	6.75	15
31	30.87	8.525	6.975	15.5
32	29.91	8.8	7.2	16
33	29.00	9.075	7.425	16.5
34	28.15	9.35	7.65	17
35	27.34	9.625	7.875	17.5
36	26.58	9.9	8.1	18
37	25.87	10.175	8.325	18.5
38	25.19	10.45	8.55	19
39	24.54	10.725	8.775	19.5
40	23.93	11	9	20

The simple logic employed above allows us to narrow down the possibilities to the elements listed in Table 1a. In all rows of this table, not only is $\Delta E_1 + \Delta E_2 = \Delta E_3$, but also $\Delta E_1 = \Delta E_2$. Data on kinesin motors show that the center of mass of the double-headed molecule moves forward 8 nm against a backward load of ~3.5−4 pN[34] (or 4 nm against a maximum

backward load of ~7 pN). In fact, when the backward load is raised above ~4 pN when kinesin is executing 8-nm forward movements, the kinesin starts stepping in the backward direction.[34] The mechanical work done during each forward step is ~30 pN nm. The energy of MgATP binding and hydrolysis steps must therefore provide ~30 pN nm or ~18 kJ mol^{-1}, and the value of $\Delta G^{0\prime}$ must measure at least 36 kJ mol^{-1}. Hence, we can neglect values of $\Delta G^{0\prime}$ that measure less than 36 kJ mol^{-1}, i.e., the upper half of Table 1a. Higher values of $\Delta G^{0\prime}$ indicate a less efficient energy transduction mechanism, and it is difficult to explain why kinesin motors start stepping backward at intermediate loads of ~4 pN nm and greater and do not continue their processive forward movement despite more free energy being available for useful mechanical work. For this reason and, further, for the sake of simplicity (e.g., occurrence of whole numbers), we can take an optimal value of $\Delta G^{0\prime}$ to be 36 kJ mol^{-1} for F-type ATPases, muscle myosin, kinesin and ncd motors, which also satisfies all of the experimental data on these systems. This leads to a subdistribution of free energy changes of ~9, ~9, and ~18 kJ mol^{-1} for the steps of ATP bond cleavage (0.3−0.4 nm), reduction of Coulombic repulsion by movement of P_i away from MgADP due to reduction of P_i binding to its site (0.4−0.6 nm), and P_i release to the medium (0.6 nm to ∞), respectively.

In the above analysis, we have employed only the standard-state free energy change values, which appear to be an adequate description for the systems considered above. We know that ~36 kJ mol^{-1} of energy is needed to drive both the motor heads of kinesin. Thus, ~18 kJ mol^{-1} is needed to drive each head. After P_i release, we obtain ~18 kJ mol^{-1}, which is sufficient to move one of the heads forward, but only ~9 kJ mol^{-1} is available to move the other head before P_i release takes place (because ~9 kJ mol^{-1} of the hydrolysis energy has already been used to weaken the binding of P_i to its site, as discussed in earlier sections). Therefore, the remaining ~9 kJ mol^{-1} has to be supplied from some other source of energy, and we postulated in previous work[27] that this balance of ~9 kJ mol^{-1} is provided by MgATP binding to the kinesin head. Hence, the required ~18 kJ mol^{-1} of free energy to drive one motor head is obtained before P_i release by the chemical steps of MgATP binding and hydrolysis acting in succession, and the other ~18 kJ mol^{-1} to drive the other head is made available upon P_i release to infinity. Because the ~9 kJ mol^{-1} of MgATP binding exactly compensates for the ~9 kJ mol^{-1} of MgATP hydrolysis energy that is utilized for reduction of P_i binding, we could satisfactorily use only the standard-state part of the total Gibbs free energy change in our calculations. This distribution of free energy changes also accounts satisfactorily for the case of rotation of the γ-shaft in F_1-ATPase and also for muscular contraction.[27] The above estimate of energy supplied by MgATP binding for motility could also be independently verified from binding affinity measurements on F_1-ATPase (available for all three β-catalytic binding sites).[27] Unfortunately, analogous measurements of the amount of free energy released by MgATP binding are not known for other molecular machines. Hence, it is not possible to independently check the estimates of the subdistribution of free energies for kinesins or unconventional myosins except by analogy. If it is found by experiments at a subsequent time that the excess free energy of MgATP binding to the track is greater than ~9 kJ mol^{-1}, then we can readily include the entire ΔG term (to which the MgATP binding also contributes). If this were the case, it would be possible to work against a higher backward load for processive machines in vitro or for muscle to lift a higher load. More experimental data on the binding energy of MgATP/MgADP to motor heads bound to actin/microtubule subunits can help to resolve this issue further.

VI. Biological Implications

The above analysis of a direct mechanism of energy transfer from ATP offers a deeper understanding of the fundamental aspects of energy transduction in biological systems and has wide-ranging biological implications. First, it explains why the free energy change of the hydrolysis step is small. This is not because the value of $\Delta G^{0\prime}$ itself is small,[6] but because there is a free energy tradeoff at the binding site.[27] In other words, the free energy released upon bond cleavage is immediately utilized to reduce the binding of a ligand to its binding site (e.g., the case of inorganic phosphate analyzed above) or to help in breaking/weakening a bond (for example, between ε and β_E in F_1-ATPase)[27] and hence is not used for the performance of useful mechanical work. However, this does not mean that the synthesis/hydrolysis step requires/releases no free energy at all, as conceived in previous mechanisms of energy transduction, such as the binding change mechanism.[6] More recent molecular mechanisms of energy transfer in ATP do not make such assumptions, and these are therefore far more in consonance with the results of the electrostatic model developed and analyzed in this work.

Second, it might not be accurate to identify the step of energy transduction as occurring during product release,[13] because the release step itself is endergonic. The torsional and RUT/RT mechanisms and the analysis carried out here show that the energy transduction per se occurs at the hydrolysis step where the two negatively charged cleavage products are generated. However, the energy between these charges remains stored as potential energy and is made available for the performance of useful work only after the binding of one of them is reduced, thereby freeing it to move away from the other. In other words, only upon product release is the Coulombic repulsion energy/stored energy of the charges harnessed for performing useful work. Hence, it appeared to several researchers that product release was directly coupled to the performance of useful work. Only upon understanding of the entire process of energy transduction, storage, and utilization in minute detail was it possible to resolve these apparent discrepancies. The torsional mechanism of energy transduction and ATP synthesis, the RUT/RT energy-storage mechanisms, and the unified theory of ATP synthesis/hydrolysis have contributed to further a deeper appreciation of these fundamental aspects of the problems of energy transfer in biological systems.

Finally, proof of the violation of both the first and the second laws of thermodynamics by previous mechanisms of energy transduction has been provided from first principles.[15,26,27] Further, previous models are grossly inconsistent with an immense amount of experimental data.[9,10,15,26,27] Previous models of ATP synthesis and muscle contraction are thus faced with the problem that, if they resolve one flaw at one place, serious flaws, drawbacks, and shortcomings arise at other places, and there seems to be no way of overcoming these deficiencies. These problems are satisfactorily resolved if researchers employ the new paradigm of the torsional/RUT/RT mechanisms and the unified theory of ATP synthesis/hydrolysis for rationalizing and interpreting their experimental data in these important fields of science. This will lead to rapid progress and result in a large number of fruitful applications in nanotechnology, systems biology, and health and disease.

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