some ancient structures have survived the onslaught of atmospheric corrosion until now. Most studies deal with decay of buildings caused by atmospheric SO_2, NO_x and HCl, completely ignoring the contributions from other atmospheric constituents, specially of biological origin. The growth of algae on rocks and concrete surfaces are widely reported[6] and only intermittent precipitation or moisture derived from dew is sufficient enough to enable motile stages of the life cycle to function. Some algae occur more frequently on acid rock surfaces, e.g. *Stigonema minutum*, *Desmid* spp., *Eunotia* spp., *Pinularia borealis*, whilst on alkaline surfaces *Aphanocapsa* spp., *Schizothrix* spp., only one *Desmid* spp. and *Melosira roeseana* are common. *Gloeocapsa kutzingiana* produces small pits in the surface when growing on calcite crystals and on marble in quarries, however, these algae occur in close association with fungal hyphae and it is not clear to what extent fungal or algal action produce pitting. Bare rock surfaces within [illegible] where sufficient light penetrates, are also colonized by *Cyanophyta*. On old church walls and grave stones, *Gloeocapsa sanguinea*, *G. kutzingiana*, *G. compacta* and *Scytonema mychrous* are very common and in many sites they are probably contributing to the breakdown of the stone. Where some water percolates over the surface, *Stichococcus* spp., *Gloeocystis* spp., *Coccomyxa* spp. and *Chlorococcus* spp., occur and these powdery green growths are found on buildings of all kinds, but are most obvious on the more ancient monuments, where time and the breakdown of the surface stone has yielded a favourable habitat. Such growths are of much concern to conservationists in Italy and Greece[6].

Thus, it seems that the deterioration of buildings in many places is mainly through the reactions of oxalic acid secreted from algae, the theoretical feasibility of which has been clearly shown in the present study. Finally it should be stated that the correct prediction of any process needs both thermodynamic and kinetic study of the systems considered. I expect that our conclusions in this paper will be supported by laboratory-controlled experimental kinetic data, which I hope to obtain soon.

1. Gouri, K. L. and Holdren, Jr., G. C., *Environ. Sci. Technol.*, 1981, **15**, 386–389.
2. Jaynes, S. M. and Cooke, R. U., *Atmos. Environ.*, 1987, **21**, 1601–1607.
3. Shrivastav, R. and Shrivastav, S., *Fluoride*, 1996, **29**, 89–94.
4. Shrivastav, R., Mathur, S. K. and Shrivastav, S., *Curr. Sci.*, 1995, **68**, 335–337.
5. Camuffo, D., Delmonte, M. and Sabbioni, C., *Water Air Soil Pollut.*, 1983, **19**, 351–359.
6. Round, F. E., *The Ecology of Algae*, Cambridge University Press, Cambridge, 1981, pp. 27–316.
7. Del Monte, M. and Sabbioni, C., *Environ. Sci. Technol.*, 1983, **17**, 518–522.
8. Fogg, G. E., *Arch. Hydrobiol. Beih. Ergebn. Linnol.*, 1971, **5**, 1–24.
9. Hellebust, J. A., *Limnol. Oceanogr.*, 1965, **10**, 192–208.
10. Nalewajka, C., *Limnol. Oceanogr.*, 1966, **11**, 1–10.
11. Weast, R. C., *Handbook of Chemistry and Physics*, CRC, Boca Raton, 1988, D35–D77.
12. Heicklen, J., *Atmospheric Chemistry*, Academic Press, New York, 1976, p. 7, 212.
13. Baedecker, P. A., Reddy, M. M., Reimann, K. J. and Sciammarella, *Atmos. Environ.*, 1992, **B26**, 147–158.
14. Sweevers, H. and Van Grieken, R., *Atmos. Environ.*, 1992, **B26**, 159–163.
15. Webb, A. H., Bawden, R. J., Busby, A. K. and Hopkins, J. N., *Atmos. Environ.*, 1992, **B26**, 165–181.

ACKNOWLEDGEMENTS. I am grateful to Prof. P. S. Satsangi, Director, Dayalbagh Educational Institute, Agra and Prof. Satya Prakash, Head, Department of Chemistry, D.E.I., Agra, for moral support and encouragement. I also thank Dr A. K. Bhatnagar, Department of Botany, D.E.I., Agra, for providing useful information on the physiology of rock algae.

Received 16 March 1998; revised accepted 10 August 1998

Mechanism of ATP synthesis by protonmotive force

Hirdesh Rohatgi, Anjanabha Saha and Sunil Nath*

Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology, Hauz Khas, New Delhi 110 016, India

ATP synthase (or F_1F_0 ATPase) is the central enzyme in biological energy conversion that is almost three billion years old and is present in the membranes of mitochondria, chloroplasts and bacteria with amazingly similar structure and function in different species. It couples proton translocation through its membrane-embedded, hydrophobic domain, F_0 to the synthesis of the energy carrier molecule, ATP in its soluble, hydrophilic headpiece, F_1. In the absence of a high-resolution structure for F_0, the complete mechanism of ATP synthesis by protonmotive force has never been elucidated. Here we propose such a mechanism. The proposed mechanism is supported by a number of independent experimental evidences.

ADENOSINE triphosphate synthase (ATP synthase or F_1F_0 ATPase) is the central enzyme in energy conversion in mitochondria, chloroplasts and bacteria, synthesizing ATP from ADP and inorganic phosphate using energy derived from a transmembrane electrochemical potential difference. The multisubunit assembly consists of a globular domain, F_1 (with composition $\alpha_3\beta_3\gamma\delta\varepsilon$) and an intrinsic membrane domain, F_0 (with composition ab_2c_{9-12}) linked by a slender stalk 4.5 nm long. The catalytic binding sites are predominantly in the β subunits at the α–β

*For correspondence. (e-mail: sunath@dbeb.iitd.ernet.in)

interface[1-4]. Energy released by the proton flux through F_0 is relayed to the catalytic sites by conformational changes through the stalk. Recent structural[1,2], biochemical[3,5] and spectroscopic[6,7] studies indicate that these conformational changes arise from intersubunit rotation in ATP synthase, making it the smallest biological rotor (rotor radius ≈ 1 nm, stator radius ≈ 5 nm) known to mankind[8]. Here we propose a unified mechanism for ATP synthesis by protonmotive force.

Each of the c subunits of F_0 has a conserved amino acid sequence which interacts with a conserved sequence of the a subunit to form a proton channel[9]. The c subunit, which functions as the rotor, has Asp-61 as the essential amino acid in each of its subunits[10,11]. Arg-210, Glu-219 and His-245 are the key amino acids in the a subunit[11,12], which acts as the stator (the residue numbers for the F_0 domain refer to *E. coli*). The exact spatial orientation of these charges is unknown and Figure 1 represents a possible geometry that can lead to generation of a unidirectional torque. The geometry of the a and c subunits is such that while c is a complete cylinder, the a subunit is part of a cylinder coaxial to c, covering two subunits of c. Thus, the interacting region of a and c subunits can be considered as the surfaces of two coaxial cylinders in close proximity to each other. They are constrained such that the only degree of freedom is for the inner cylinder (c subunit) to rotate with respect to the fixed outer cylinder (a subunit).

The negatively charged Asp-61 must be protonated when exposed to the membrane and unprotonated at the a–c interface. The electrostatic potential field defined by the charge distribution will have many equilibria (one for each subunit) and when both Asp-61 residues are unprotonated, the system is at equilibrium. When a proton enters the proton channel, it neutralizes the leading Asp-61 residue. Now the positively charged His-245 attracts the trailing unprotonated Asp-61, disturbing the equilibrium and causing the inner cylinder to rotate to a new local equilibrium position (Figure 1). Thus the leading Asp-61 moves into the membrane and a new protonated Asp-61 enters the interface. The charge distribution is such that each successive disruption of equilibrium causes the cylinder to rotate in a unidirectional mode. Rotation in the reverse direction is prevented by the large free energy barrier to transport an unprotonated Asp-61 from the interface into the hydrophobic membrane environment. The positive Arg-210 ensures that only the leading Asp-61 is protonated and along with the negative Glu-219, induces the proton on the new Asp-61 to jump into the matrix (Figure 1). Thus, according to the mechanism proposed above, the His-245 is essential for proton translocation, while the Arg-210 and Glu-219 residues, though not essential for proton translocation, are important for the efficient working of the c rotor.

The above points also illustrate how our proposed mechanism of torque generation differs from the mechanisms proposed by other workers in the field. In the work of Elston *et al.*[13], the driving force for proton translocation is biased diffusion due to the proton gradient, while Vik and Antonio[11] consider the formation and breaking of a hydrogen bond during rotation. As opposed to these treatments, electrostatics is the driving force in our mechanism. In the case of stator amino acid residues, we consider His-245 essential for proton translocation (or at least, it should tolerate few substitutions), and Arg-210 and Glu-219 important, but not essential. On the other hand, Elston *et al.*[13] claim that Arg-210 is the essential residue but assign no role to the other two residues, while Vik and Antonio[11] consider all the three amino acids to be essential for proton translocation.

The torque generated in the F_0 subunit by the above mechanism is transmitted to the connecting stalk (comprising of γ and ε subunits of F_1), which is attached to the center of the c rotor[14-16]. Since the c subunit has 9–12 copies, the torque generation is quantized in steps of 9–12; however the three-fold symmetry of F_1 indicates that this number should be either 9 or 12. The binding change mechanism along with the crystal structure of F_1 and the decay of the polarization anisotropy parameter imply that the γ subunit rotates in steps of three[1-3,6,7].

The nucleotide-free $\alpha_3\beta_3$ subcomplex of ATP synthase is a symmetric trimer[2] but at any instant of time, the three catalytic sites possess different conformations as formulated in the binding change mechanism and confirmed in the crystal structure[1-3]. One of the sites in the crystal structure binds the ATP analogue AMP-PNP and is designated as β_{TP}; another site binds ADP and is denoted by β_{DP} while the third site is empty and distorted and is called β^1_E. The γ and ε subunits rotate relative to $\alpha_3\beta_3$ and we propose for the first time that the unique ε subunit (in *E. coli*) is always closest to

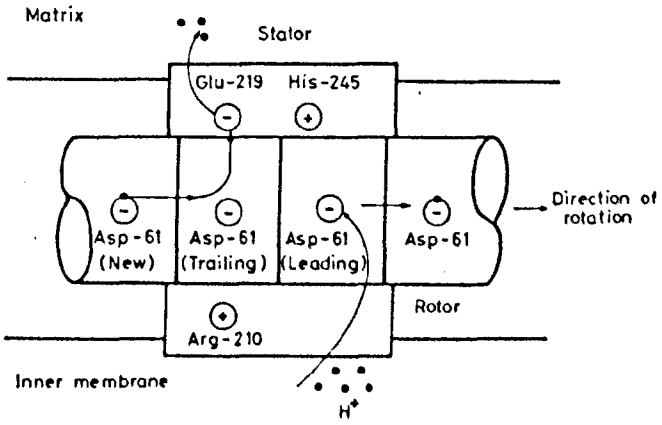

Figure 1. Charge geometry for torque generation from protonmotive force in ATP synthase. The essential amino acid residues are shown. The stator is stationary while the rotor moves from left to right. The path followed by the protons is indicated by arrows.

the catalytic site with the *least* affinity for ATP. As the γ and ϵ subunits rotate, their interactions with a particular catalytic binding site change, thereby causing conformational changes at that site. The asymmetry in the γ-subunit can be understood by considering it to have three faces interacting asymmetrically (based on the number of interactions) with the catalytic sites. The amino acids responsible for these interactions are strictly conserved in mitochondria and bacteria. In a β subunit which has no interactions with γ and ϵ, both ADP and P_i can diffuse in easily. There exists a binding site for ADP, but there is insufficient space to accommodate both ADP and P_i as separate entities. The Mg^{2+} ions stabilize the α- and β-phosphates of ADP. We propose that the binding of ADP and P_i takes place in β_{TP}. One face of the γ subunit (Lys-87 and Lys-90) interacts with the DELSEED sequence (394–400) in β^1_{TP} and causes the binding site to open slightly, which allows the P_i to come close to ADP (the residue numbers for the F_1 domain refer to mitochondria). This can be considered as the loose site. The γ subunit now rotates and the above interactions break. As the new face of γ has no interactions with the catalytic site, it goes back to its original position and the conformation of the site becomes tight (β_{DP}). This forces the P_i to move towards ADP and attach to it; the pentacoordinate transition state formed is stabilized by the movement of the α-Arg 373 towards the β-phosphate moiety. Further, Mg^{2+} moves locally from a position which stabilized the α- and β-phosphates to a position where it can stabilize the β- and γ-phosphates of the transition state. The transition state leads to spontaneous formation of ATP along with the release of a water molecule. This is followed by the conformation β_E in which the C terminal of the γ-subunit (Arg-254 and Gln-255) interacts with the Asp-316 and Asp-319 of β_E. Further, Ser-108 of the rotating ϵ subunit interacts covalently[14,17] with Glu-381 of β_E as a result of which the lower half of β_E is hinged out, leading to the opening of the catalytic site and release of the bound ATP in this open conformation. The upper half of β_E is held in place by its interaction with the γ subunit. Rotation of the γ and ϵ subunits causes these interactions to break, leading to the re-establishment of the β_{TP} conformation and the entire cycle repeats.

The mechanism proposed above is supported by a number of *independent* experimental evidences. The essential amino acid residues which we use in formulating our charge geometry have all been identified by site-directed mutagenesis studies[9–12]. The interactions of the γ and ϵ subunits with the c subunit (which would be essential for the transmission of torque) are clearly inferred from the recently determined crystal structure of the ϵ-subunit of *E. coli*[14]. There exists strong structural, biochemical and spectroscopic evidence for intersubunit rotation[1–7]; moreover, rotation in F_1ATPase has been observed directly in a recent work[8]. The interactions of the γ subunit with the β subunits are consistent with the crystal structures of F_1ATPase reported in the literature[1,2]. Recent crosslinking studies lend additional support[17]. Finally, the mechanism proposed here is in complete agreement with the binding change mechanism[3].

In summary, we have presented the *first, unified* mechanism for the synthesis of ATP by protonmotive force in ATP synthase, the smallest known molecular machine.

1. Abrahams, J. P., Leslie, A. G. W., Lutter, R. and Walker, J. E., *Nature*, 1994, **370**, 621–628.
2. Shirakihara, Y., Leslie, A. G. W., Abrahams, J. P., Walker, J. E., Ueda, T., Sekimoto, Y., Kambara, M., Saika, K., Kagawa, Y. and Yoshida, M., *Structure*, 1997, **5**, 825–836.
3. Boyer, P. D., *Biochim. Biophys. Acta*, 1993, **1140**, 215–250.
4. Senior, A. E., *Physiol. Rev.*, 1988, **68**, 177–231.
5. Zhou, Y., Duncan, T. M., Bulygin, V. V., Hutcheon, M. L. and Cross, R. L., *Biochim. Biophys. Acta*, 1996, **1275**, 96–100.
6. Sabbert, D., Engelbrecht, S. and Junge, W., *Nature*, 1996, **381**, 623–625.
7. Sabbert, D. and Junge, W., *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 2312–2317.
8. Noji, H., Yasuda, R., Yoshida, M. and Kinosita, K., *Nature*, 1997, **386**, 299–302.
9. Cox, G. B., Fimmel, A. L., Gibson, F. and Hatch, L., *Biochim. Biophys. Acta*, 1986, **849**, 62–69.
10. Fraga, D., Hermolin, J. and Fillingame, R. H., *J. Biol. Chem.*, 1994, **269**, 2562–2567.
11. Vik, S. B. and Antonio, B. J., *J. Biol. Chem.*, 1994, **269**, 30364–30369.
12. Eya, S., Maeda, M. and Futai, M., *Arch. Biochem. Biophys.*, 1991, **284**, 71–77.
13. Elston, T., Wang, H. and Oster, G., *Nature*, 1998, **391**, 510–513.
14. Uhlin, U., Cox, G. B. and Guss, J. M., *Structure*, 1997, **5**, 1219–1230.
15. Zhang, Y., Oldenburg, M. and Fillingame, R. H., *J. Biol. Chem.*, 1994, **269**, 10221–10224.
16. Zhang, Y. and Fillingame, R. H., *J. Biol. Chem.*, 1995, **270**, 24609–24614.
17. Aggeler, R., Haughton, M. A. and Capaldi, R. A., *J. Biol. Chem.*, 1995, **270**, 9185–9191.

ACKNOWLEDGEMENTS. S.N. acknowledges financial support for this research from the DST (Grant SR/OY/GB-26/93) and the All-India Council for Technical Education (Grant 1-52/CD/CA/95-96).

Received 31 January 1998; revised accepted 20 August 1998