4

ATP is generated by photosynthesis

Chapter 3 discussed the transport of protons across a thylakoid membrane by photosynthetic electron transport and how, in this way, a proton gradient is generated. This chapter describes how this proton gradient is utilized for the synthesis of ATP.

In 1954 Daniel Arnon (Berkeley) discovered that upon illumination suspended thylakoid membranes synthesize ATP from ADP and inorganic phosphate. This process is called **photophosphorylation**. Further experiments showed that photophosphorylation is coupled to the generation of NADPH. This result was unexpected, as at that time it was generally believed that the synthesis of ATP in chloroplasts, as in mitochondria, was driven by an electron transport from NADPH to oxygen. It soon became apparent, however, that the mechanism of photophosphorylation coupled to photosynthetic electron transport was very similar to that of ATP synthesis coupled to electron transport of mitochondria, termed **oxidative phosphorylation** (section 5.6).

In 1961 Peter Mitchell (Edinburgh) postulated in his **chemiosmotic hypothesis** that during electron transport a proton gradient is formed, and that it is the **proton motive force** of this gradient that drives the synthesis of ATP. At first this revolutionary hypothesis was strongly opposed by many workers in the field, but in the course of time, experimental results of many researchers supported the chemiosmotic hypothesis, which is now fully accepted. In 1978 Peter Mitchell was awarded the Nobel Prize in Chemistry for this hypothesis.

4.1 A proton gradient serves as an energy-rich intermediate state during ATP synthesis

Let us first ask: How much energy is actually required in order to synthesize ATP?

The free energy for the synthesis of ATP from ADP and phosphate is calculated from the van't Hoff equation:

$$\Delta G = \Delta G^{0'} + RT \ln \frac{[ATP]}{[ADP] \cdot [P]} \tag{4.1}$$

The standard free energy for the synthesis of ATP is:

$$\Delta G^{0'} = +30.5\,\text{kJ/mol} \tag{4.2}$$

The concentrations of ATP, ADP, and phosphate in the chloroplast stroma are very much dependent on metabolism. Typical concentrations are:

$$\text{ATP} = 2.5 \cdot 10^{-3}\ \text{mol/L};\ \text{ADP} = 0.5 \cdot 10^{-3}\ \text{mol/L};\ \text{P} = 5 \cdot 10^{-3}\ \text{mol/L}$$

When these values are introduced into equation 4.1 ($R = 8.32\,\text{J/mol} \cdot \text{K}$, $T = 298\,\text{K}$), the energy required for synthesis of ATP is evaluated as:

$$\Delta G = +47.8\,\text{kJ/mol} \tag{4.3}$$

This value is, of course, variable because it depends on the metabolic conditions. For further considerations an average value of 50 kJ/mol will be employed for ΔG_{ATP}.

The transport of protons across a membrane can have different effects. If the membrane is permeable to counter ions of the proton (e.g., a chloride ion (Fig. 4.1A)), the charge of the proton will be compensated, since each transported proton will pull a chloride ion across a membrane. This is how a proton concentration gradient can be generated. The free energy for the transport of protons from A to B is:

$$\Delta G = RT \ln \frac{[H^+]_B}{[H^+]_A} \quad [\text{J/mol}] \tag{4.4}$$

If the membrane is impermeable for counter ions (Fig. 4.1B), a charge compensation for the transported proton is not possible. In this case, the

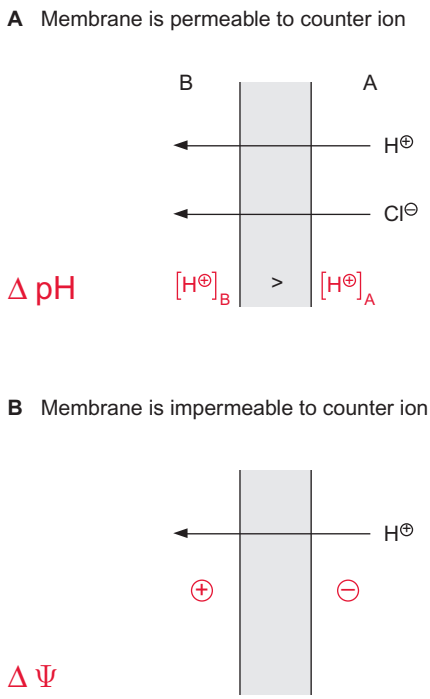


Figure 4.1 A. Transport of protons through a membrane. Permeablity to a counter ion such as chloride results in the formation of a proton gradient. B. When the membrane is impermeable to a counter ion, proton transport results in the formation of a membrane potential.

transfer of only a few protons across the membrane results in the formation of a membrane potential $\Delta\Psi$, measured as the voltage difference across the membrane. By convention, $\Delta\Psi$ is positive when a cation is transferred in the direction of the more positive region. Voltage and free energy are connected by the following equation:

$$\Delta G = mF \cdot \Delta\Psi \tag{4.5}$$

where m is the charge of the ion (in the case of a proton = 1), and F is the Faraday constant, 96,485 A s/mol.

Proton transport across a biological membrane leads to the formation of a proton concentration gradient and a membrane potential. The free energy for the transport of protons from A to B therefore consists of the sum of the free energies for the generation of the H^+ concentration gradient and the membrane potential:

$$\Delta G = RT \ln \bullet \frac{[H^+]_B}{[H^+]_A} + F\Delta\Psi \tag{4.6}$$

In chloroplasts, the energy stored in a proton gradient corresponds to the change of free energy during the flux of protons from the lumen into the stroma.

$$\Delta G = RT \ln \frac{[H^+]_S}{[H^+]_L} + F\Delta\Psi \tag{4.7}$$

where S = stroma, L = lumen, and $\Delta\Psi$ = voltage difference stroma-lumen.

The conversion of the natural logarithm into the decadic logarithm yields:

$$\Delta G = 2.3 \cdot RT \log \frac{[H^+]_S}{[H^+]_L} + F\Delta\Psi \tag{4.8}$$

The logarithmic factor is the negative pH difference between lumen and stroma:

$$\log[H^+]_S - \log[H^+]_L = -\Delta\mathrm{pH} \tag{4.9}$$

A rearrangement yields:

$$\Delta G = -2.3RT \bullet \Delta\mathrm{pH} + F\Delta\Psi \tag{4.10}$$

At 25°C: $2.3 \cdot RT = 5{,}700\,\mathrm{J/mol}$

Thus: $$\Delta G = -5{,}700\Delta\mathrm{pH} + F\Delta\Psi \quad [\mathrm{J/mol}] \tag{4.11}$$

The expression $\frac{\Delta G}{F}$ is called **proton motive force** (PMF) (unit in volts):

$$\frac{\Delta G}{F} = PMF = -\frac{2.3RT}{F} \cdot \Delta pH + \Delta\Psi \quad [\mathrm{V}] \tag{4.12}$$

At 25°C: $\frac{2.3RT}{F} = 0.059\,\mathrm{V}$

Thus: $$\mathrm{PMF} = -0.059 \cdot \Delta\mathrm{pH} + \Delta\Psi \quad [\mathrm{V}] \tag{4.13}$$

Equation 4.13 is of general significance for electron transport-coupled ATP synthesis. In mitochondrial oxidative phosphorylation, the PMF is primarily the result of a membrane potential. In chloroplasts, on the other

hand, the membrane potential does not contribute much to the PMF, since the PMF is almost entirely due to the concentration gradient of protons across the thylakoid membrane. In illuminated chloroplasts, a ΔpH across the thylakoid membrane of about 2.5 can be measured. Introducing this value into equation 4.11 yields:

$$\Delta G = -14.3\,\text{kJ/mol}$$

A comparison of this value with ΔG for the formation of ATP (50 kJ/mol) suggests that at least four protons are required for the ATP synthesis from ADP and phosphate.

4.2 The electron chemical proton gradient can be dissipated by uncouplers to heat

Photosynthetic electron transport from water to $NADP^+$ is coupled with photophosphorylation. Electron transport occurs only if ADP and phosphate are present as precursor substances for ATP synthesis. When an **uncoupler** is added, electron transport proceeds at a high rate in the absence of ADP; electron transport is then uncoupled from ATP synthesis. Therefore, in the presence of an uncoupler, ATP synthesis is abolished.

The chemiosmotic hypothesis explains the effect of uncouplers (Fig. 4.2). Uncouplers are amphiphilic compounds, soluble in both water and lipids. They are able to permeate the lipid phase of a membrane by **diffusion** and in this way to transfer a proton or an alkali ion across the membrane, thus eliminating a proton concentration gradient or a membrane potential, respectively. In the presence of an uncoupler a proton gradient is absent, but protons are transported by ATP synthase from the stroma to the thylakoid lumen. This proton transport costs energy: ATP is hydrolyzed to ADP and phosphate. This is the reason why uncouplers cause an ATP hydrolysis (**ATPase**).

Figure 4.2A shows the effect of the uncoupler carbonylcyanide-*p*-trifluormethoxyphenylhydrazone (**FCCP**), which is a weak acid. FCCP diffuses in the undissociated (protonated) form from the compartment with a high proton concentration (on the left in Fig. 4.2A), through the membrane into the compartment with a low proton concentration, where it finally dissociates into a proton and the FCCP anion**.** The proton remains and the FCCP anion returns by diffusion to the other compartment, where it is protonated again. In this way the presence of FCCP at a concentration of only

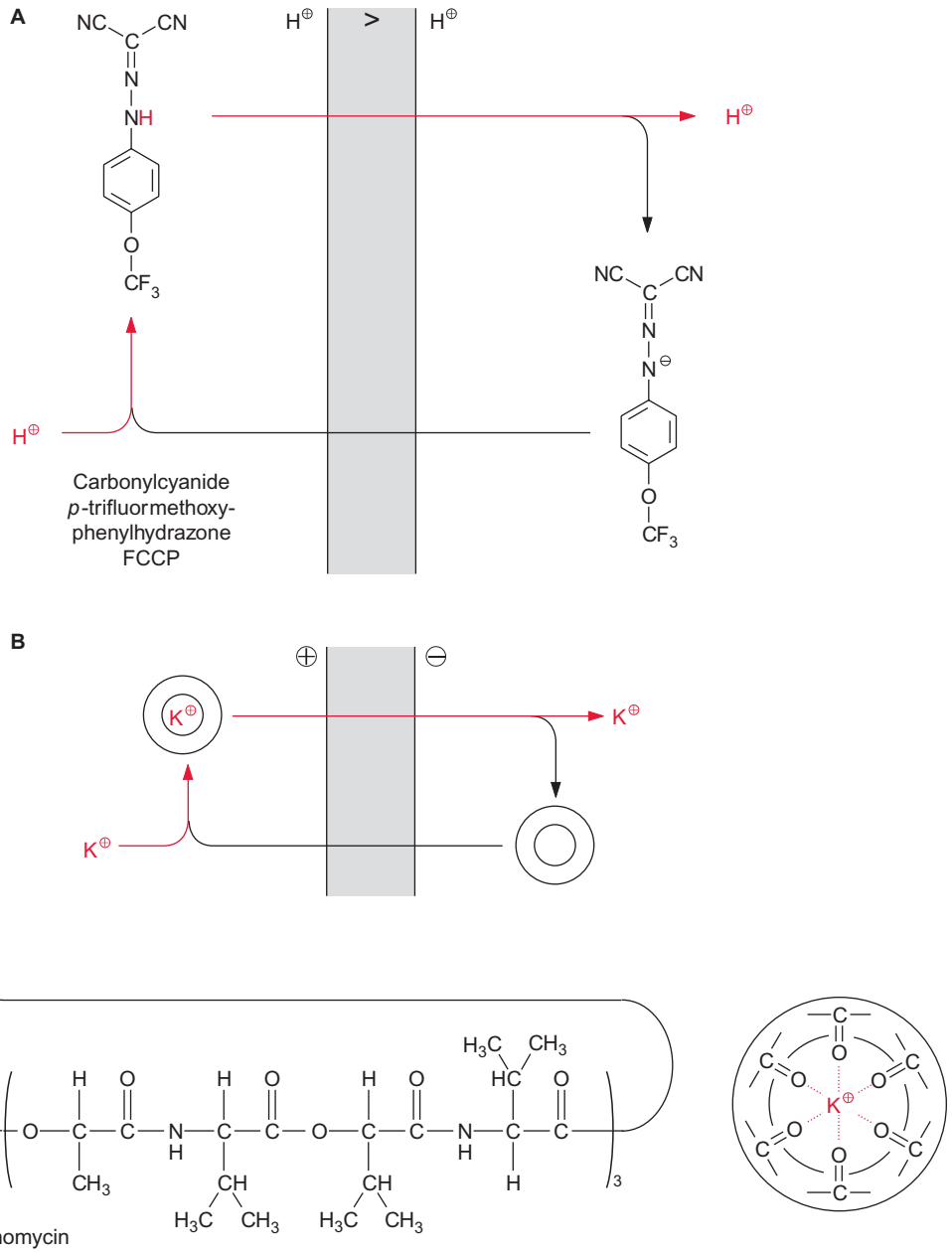
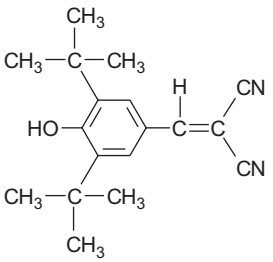


Figure 4.2 The proton motive force of a proton gradient is eliminated by uncouplers. A. The hydrophobicity of FCCP allows the diffusion through a membrane in the protonated form as well as in the deprotonated form. This uncoupler, therefore, eliminates a proton gradient by proton transfer. B. Valinomycin, an antibiotic with a cyclic structure, folds to a hydrophobic spherical molecule, which is able to bind K^+ ions in the interior. Loaded with K^+ ions, valinomycin can diffuse through a membrane. In this way valinomycin can eliminate a membrane potential by transferring K^+ ions across a membrane.

$7 \cdot 10^{-8}$mol/L results in complete dissipation of the proton gradient. The substance **SF 6847** (3.5-Di (*tert*-butyl)-4-hydroxybenzyldimalononitril) (Fig. 4.3) has an even higher uncoupling effect. Uncouplers such as FCCP or SF 6847, which transfer protons across a membrane, are called **protonophores**.

In addition to the protonophores, there is a second class of uncouplers, termed **ionophores**, which are able to transfer alkali cations across a membrane and thus dissipate a membrane potential. **Valinomycin**, an antibiotic from *Streptomyces*, is such an ionophore (Fig. 4.2B). Valinomycin is a cyclic molecule containing the sequence (L-lactate)-(L-valine)-(D-hydroxyisovalerate)-(D-valine) three times. Due to its hydrophobic outer surface, valinomycin is able to diffuse through a membrane. Oxygen atoms directed towards the inside of the valinomycin molecule form the binding site for dehydrated Rb^+ and K^+ ions. Na^+ ions because of their small size are only very loosely bound. When K^+ ions are present, the addition of valinomycin results in the elimination of the membrane potential. The ionophore **gramicidine**, not discussed here in detail, is also a polypeptide antibiotic. Gramicidine incorporates into membranes and forms a transmembrane ion channel by which both alkali cations and protons can diffuse through the membrane.



SF 6847

Figure 4.3 Di(*tert*-butyl)-4-hydroxybenzyl malononitrile (SF6847) is an especially effective uncoupler. Only 10^{-9}mol/L of this compound is sufficient to completely dissipate a proton gradient across a membrane. This uncoupling is based on the permeation of the protonated and deprotonated molecule through the membrane, as shown in Figure 4.2A for FCCP.

The chemiosmotic hypothesis was proved experimentally

In 1966 the American scientist André Jagendorf presented conclusive evidence for the validity of the chemiosmotic hypothesis involved in chloroplast photophosphorylation (Fig. 4.4). He incubated thylakoid membranes in an acidic medium of pH 4 in order to acidify the thylakoid lumen by unspecific uptake of protons. In a next step he added inorganic phosphate and ADP to the thylakoid suspension and then increased the pH of the medium to pH 8 by adding an alkaline buffer. This led to the sudden generation of a proton gradient of $\Delta pH = 4$, and for a short time ATP was synthesized. Since this experiment was carried out in the dark, it presented evidence that synthesis of ATP in chloroplasts can be driven without illumination just by a pH gradient across a thylakoid membrane.

4.3 H^+-ATP synthases from bacteria, chloroplasts, and mitochondria have a common basic structure

How is the energy of the proton gradient utilized to synthesize ATP?

A proton coupled ATP synthase (H^+-ATP synthase) is not unique to the chloroplast. It evolved during an early stage of evolution and occurs in

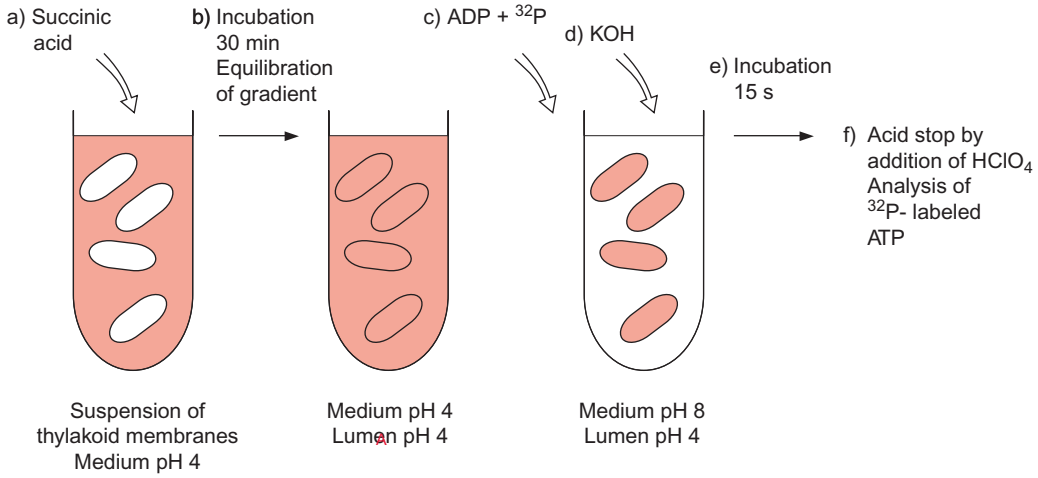


Figure 4.4 Thylakoid membranes can synthesize ATP in the dark by an artificially formed proton gradient. In a suspension of thylakoid membranes, the pH in the medium is lowered to 4.0 by the addition of succinic acid (a). After incubation for about 30 minutes, the pH in the thylakoid lumen is equilibrated with the pH of the medium due to a slow permeation of protons across the membrane (b). The next step is to add ADP and phosphate, the latter being radioactively labeled by the isotope ^{32}P (c). Then the pH in the medium is raised to 8.0 by adding KOH (d). In this way a ΔpH of 4.0 is generated between the thylakoid lumen and the medium, and this gradient drives the synthesis of ATP from ADP and phosphate. After a short time of reaction (e), the mixture is denatured by the addition of perchloric acid, and the amount of radioactively labeled ATP formed in the deproteinized extract is determined. (After Jagendorf, 1966.)

its basic structure in bacteria, chloroplasts, and mitochondria. In bacteria this enzyme catalyzes not only ATP synthesis driven by a proton gradient, but also (in a reversal of this reaction) the transport of protons against the concentration gradient at the expense of ATP. This was probably the original function of the enzyme. In some bacteria an ATPase homologous to the H^+-ATP synthase functions as an ATP-dependent Na^+ transporter.

Our present knowledge about the structure and function of the H^+-ATP synthase derives from investigations of mitochondria, chloroplasts, and bacteria. By 1960 progress in electron microscopy led to the detection of small particles, which were attached by stalks to the inner membranes of mitochondria and the thylakoid membranes of chloroplasts. These particles occur only at the matrix or stromal side of the corresponding membranes. By adding urea, Ephraim Racker and coworkers (Cornell University, USA), succeeded in removing these particles from mitochondrial membranes. The isolated particles catalyzed the hydrolysis of ATP to ADP and phosphate. Racker called them **F1-ATPase**. Mordechai Avron (Rehovot, Israel)

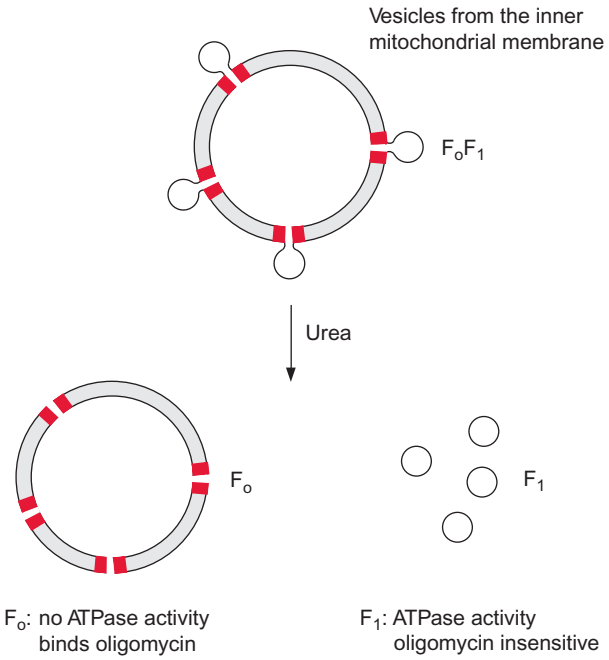


Figure 4.5 Vesicles prepared by ultrasonic treatment of mitochondria contain functionally intact H^+-ATP synthase. The soluble factor F_1 with ATPase function is removed by treatment with urea. The oligomycin binding factor F_o remains in the membrane.

showed that the corresponding particles from chloroplast membranes also have ATPase activity.

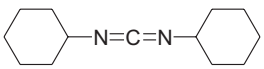
Vesicles containing F_1 particles could be prepared from the inner membrane of mitochondria. These membrane vesicles synthesized ATP during respiration, and as in intact mitochondria (section 5.6), the addition of uncouplers resulted in an increased ATPase activity. The uncoupler-induced ATPase activity, as well as ATP synthesis performed by these vesicles, was found to be inhibited by the antibiotic **oligomycin**. Mitochondrial vesicles where the F_1 particles had been removed showed no ATPase activity but were highly permeable for protons. This proton permeability was eliminated by adding oligomycin. In contrast, the ATPase activity of the removed F_1 particles was not affected by oligomycin. These and other experiments showed that the H^+-ATP synthase of the mitochondria consists of two parts:

1. A soluble factor 1 (F_1) that catalyzes the synthesis of ATP; and
2. A membrane-bound factor enabling the flux of protons through the membrane to which oligomycin is bound.

Racker designated this factor **Fo** (O, oligomycin) (Fig. 4.5). Basically the same result was found for H^+-ATP synthases of chloroplasts and bacteria, with the exception that the H^+-ATP synthase of chloroplasts is not

Table 4.1: Compounds of the F-ATP synthase from chloroplasts. Nomenclature as in *E. coli* F-ATP synthase

Subunits	Number in F_oF_1-molecule	Molecular mass (kDa)	Encoded in
F_1:α	3	55	Plastid genome
β	3	54	Plastid genome
γ	1	36	Nuclear genome
δ	1	21	Nuclear genome
ε	1	15	Plastid genome
F_o:a	1	27	Plastid genome
b	1	16	Nuclear genome
b′	1	21	Plastid genome
c	12	8	Plastid genome



Dicyclohexylcarbodiimide
DCCD

Figure 4.6
Dicyclohexylcarbodiimide (DCCD), an inhibitor of the F_o part of F-ATP synthase.

inhibited by oligomycin. Despite this, the membrane part of the chloroplastic ATP synthase is also designated as F_o. The H^+-ATP synthases of chloroplasts, mitochondria, and bacteria, as well as the corresponding H^+- and Na^+-ATPases of bacteria, are collectively termed **F-ATP synthases** or **F-ATPases**. The terms F_oF_1-ATP synthase and F_oF_1-ATPase are also used.

F_1, after removal from the membrane, is a soluble oligomeric protein with the composition $\alpha_3\beta_3\gamma\delta\varepsilon$ (Table 4.1). This composition has been found in chloroplasts, bacteria, and mitochondria.

F_o is a strongly hydrophobic protein complex that can be removed from the membrane only by detergents. Dicyclohexylcarbodimide (**DCCD**) (Fig. 4.6) binds to the F_o embedded in the membrane, and thus closes the proton channel. In chloroplasts four different subunits have been detected as the main constituents of F_o and are named a, b, b′, and c (Table 4.1, Fig. 4.7). Subunit c, probably occurring in the chloroplastic F_o in 12–14 copies, contains two transmembrane helices and is the binding site for DCCD. The c subunits appear to form a cylinder, which spans the membrane. On the outside of the cylinder spanning the membrane, the subunits a, b, and b′ are arranged, whereby b and b′ are in contact with the F_1 part via subunit δ. Subunits γ and ε form the central connection between F_1 and F_o.

Whereas the structure of the F_o part is still somewhat hypothetical, the structure of the F_1 part has been thoroughly investigated (Fig. 4.7). The F_1

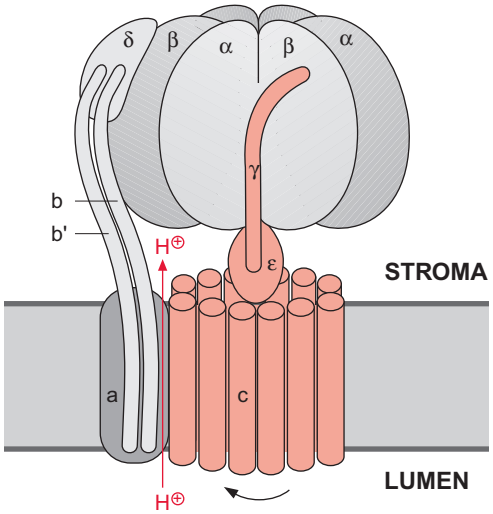


Figure 4.7 Scheme of the structure of an F-ATP synthase. The structure of the F_1 subunit concurs with the results of X-ray analysis discussed in the text. (After Junge.)

particles are so small that details of their structure are not visible on a single electron micrograph. However, details of the structure can be resolved if a very large number of F_1 images obtained by electron microscopy are subjected to a **computer-aided image analysis**. Figure 4.8 shows a delineated image of F-ATP synthase from chloroplasts. In the side projection, the stalk connecting the F_1 part with the membrane can be recognized. Using more refined picture analysis (not shown here), two stems, one thick and the other thin, were found between F_1 and F_o. In the vertical projection, a hexagonal array is to be seen, corresponding to an alternating arrangement of α- and β-subunits. Investigations of the isolated F_1 protein showed that an F_oF_1 protein has three catalytic binding sites for ADP or ATP. One of these binding sites is occupied by very tightly bound ATP, which is released only when energy is supplied from the proton gradient.

X-ray structure analysis of the F_1 part of ATP synthase yields an insight into the machinery of ATP synthesis

In 1994 the group of John Walker in Cambridge (England) succeeded in analyzing the three-dimensional structure of the F_1 part of ATP synthase. Crystals of F_1 from beef heart mitochondria were used for this analysis. Prior to crystallization, the F_1 preparation was loaded with a mixture of ADP and an ATP analogue (5′adenylyl-imidodiphosphate, AMP-PNP). This ATP analogue differs from ATP in that the last two phosphate residues are connected by an N atom. It binds to the ATP binding site as ATP,

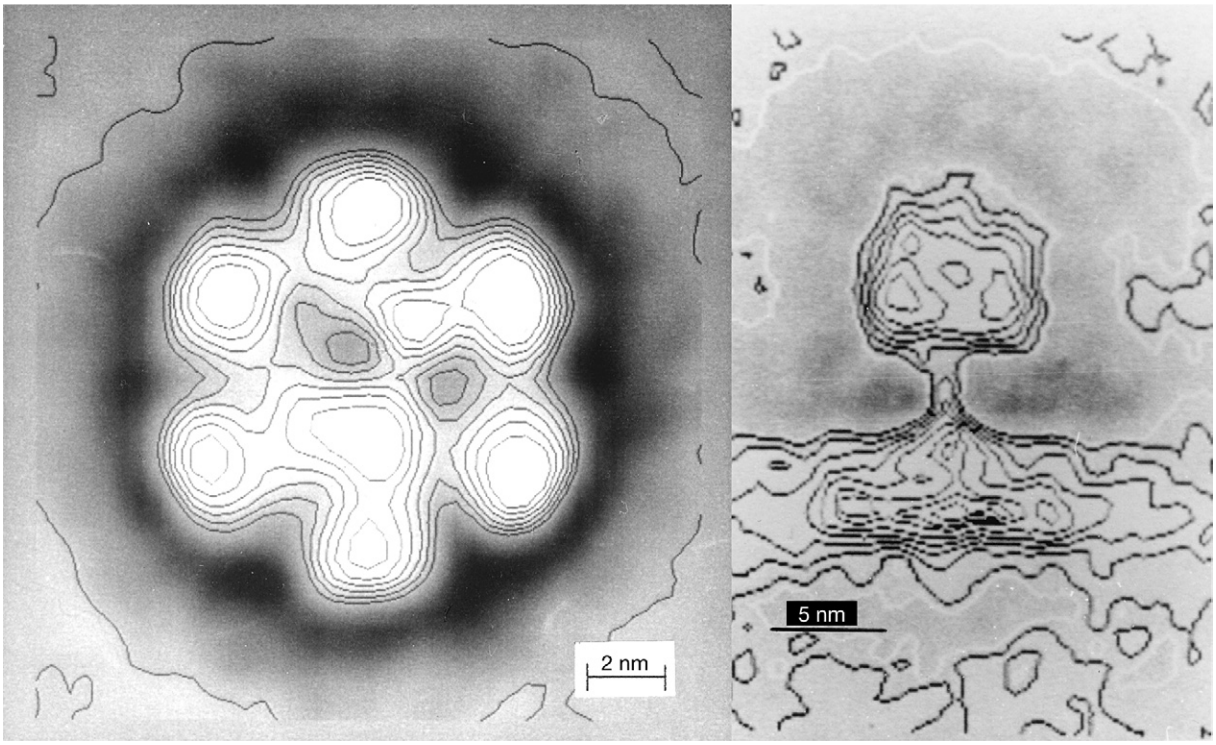


Figure 4.8 Averaged image of 483 electromicrographs of the F-ATP synthase from spinach chloroplasts. A. Vertical projection of the F_1 part. A hexameric structure reflects the alternating ($\alpha\beta$)-subunits. B. Side projection, showing the stalk connecting the F_1 part with the membrane. (By P. Graeber, Stuttgart.)

but cannot be hydrolyzed by the ATPase. The structural analysis confirmed the alternating arrangement of the α- and β-subunits (Figs. 4.7 and 4.9). One α- and one β-subunit form a unit comprising a binding site for one adenine nucleotide. The β-subunit is primarily involved in the synthesis of ATP. In the F_1 crystal investigated, one ($\alpha\beta$)-unit contained one ADP, the second the ATP analogue, whereas the third ($\alpha\beta$)-subunit was empty. These differences in nucleotide binding were accompanied by conformational differences of the three β-subunits (Fig. 4.9). The γ-subunit is arranged **asymmetrically**, protrudes through the center of the F_1 part, and is bent to the side of the ($\alpha\beta$)-unit loaded with ADP (Figs. 4.7 and 4.9). This asymmetry enlightens the function of the F_1 part of ATP synthase. Some general considerations about ATP synthase will be made before the function is explained in more detail.

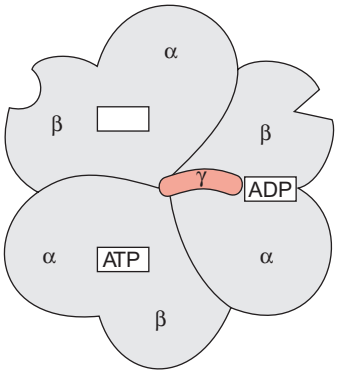


Figure 4.9 Schematic presentation of the vertical projection of the F_1 part of the F-ATP synthase. The enzyme contains three nucleotide binding sites, each consisting of an α-subunit and a β-subunit. Each of the three β-subunits occurs in a different conformation. The γ-subunit in the center, vertical to the viewer, is bent to the α- and β-subunit loaded with ADP. This representation corresponds to the results of X-ray structure analysis by Walker and coworkers mentioned in the text.

4.4 The synthesis of ATP is effected by a conformation change of the protein

For the reaction:

$$\text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{ADP} + \text{Phosphate}$$

the standard free energy is:

$$\Delta G^{\circ\prime} = -30.5\,\text{kJ/mol}$$

Because of its high free energy of hydrolysis, ATP is regarded as an energy-rich compound. It should be noted, however, that the standard value $\Delta G^{\circ\prime}$ has been determined for an aqueous solution of 1 mol of ATP, ADP, and phosphate per liter, respectively, corresponding to a water concentration of 55 mol/L. If the concentration of water was only 10^{-4} mol/L, the ΔG for ATP hydrolysis would be +2.2 kJ/mol. This means that at very low concentrations of water the reaction proceeds towards the synthesis of ATP. This example demonstrates that **in the absence of water the synthesis of ATP does not require the uptake of energy**.

The catalytic site of an enzyme can form a reaction site where water is excluded. Catalytic sites are often located in a hydrophobic area of the enzyme protein in which the substrates are bound in the absence of water. Thus, with ADP and P tightly bound to the enzyme, the synthesis of ATP could proceed spontaneously without requiring energy (Fig. 4.10). This has been proved for H^+-ATP synthase. Since the step of ATP synthesis itself does proceed without the uptake of energy, the amount of energy required to form ATP from ADP and phosphate in the aqueous phase has to be otherwise consumed, e.g., for the removal of the tightly bound newly synthesized

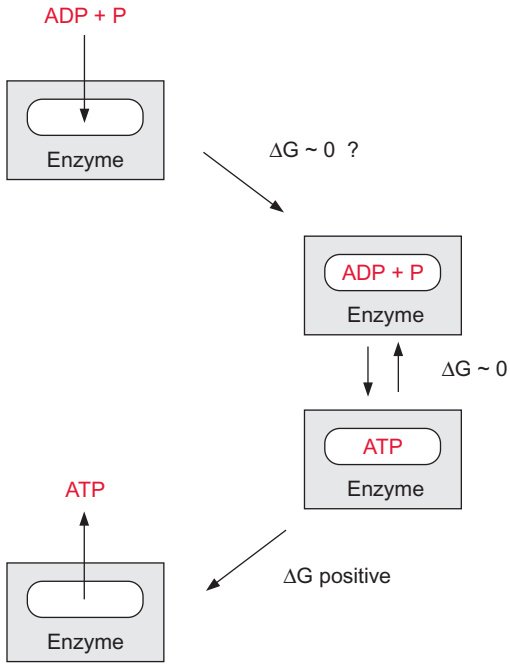


Figure 4.10 In the absence of H_2O, ATP synthesis can occur without the input of energy. In this case, the energy required for ATP synthesis in an aqueous solution has to be spent on binding ADP and P and/or on the release of the newly formed ATP. From available evidence, the latter case is more likely.

ATP from the binding site. This could occur by an energy-dependent conformation change of the protein.

In 1977 Paul Boyer (USA) put forward the hypothesis that the **three identical sites of the F1 protein alternate in their binding properties** (Fig. 4.11). One of the binding sites is present in the L form, which binds ADP and phosphate loosely but is not catalytically active. A second binding site, T, binds ADP and ATP tightly and is catalytically active. The third binding site, O, is open, binds ADP and ATP only very loosely, and is catalytically inactive. According to this ***"binding change" hypothesis***, the synthesis of ATP proceeds in a cycle. First, ADP and phosphate are bound to the loose binding site, L. A conformational change of the F_1 protein converts site L to a binding site T, where ATP is synthesized from ADP and phosphate in the absence of water. The ATP formed remains tightly bound. Another conformational change converts the binding site T to an open binding site O, and the newly formed ATP is released. A crucial point of this hypothesis is that with the conformational change of the F_1 protein, driven by the energy of the proton gradient, the conformation of each of the three catalytic sites is converted simultaneously to the next conformation

$$(L \rightarrow T \qquad T \rightarrow O \qquad O \rightarrow L)$$

The results of X-ray analysis, shown above, support the binding change hypothesis. The evaluated structure clearly shows that the three subunits

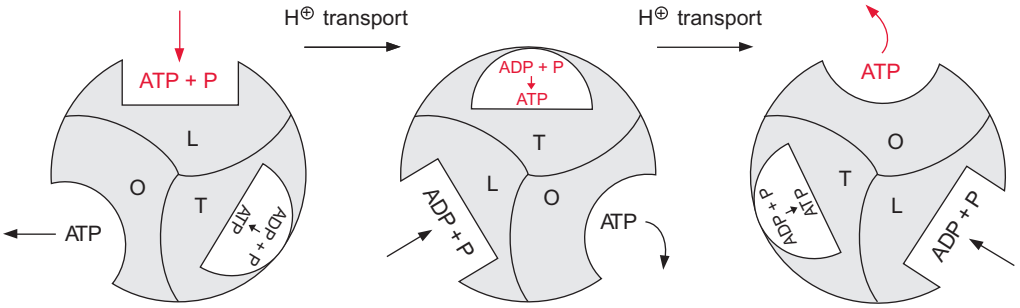


Figure 4.11 ATP synthesis by the binding change mechanism as proposed by Boyer. The central feature of this postulated mechanism is that synthesis of ATP proceeds in the F_1 complex by three nucleotide binding sites, which occur in three different conformations: conformation L binds ADP and P loosely, T binds ADP and P tightly and catalyzes the ATP formation; the ATP thus formed is tightly bound. The open form, O, releases the newly formed ATP. The flux of protons through the F-ATP synthase, as driven by the proton motive force, results in a concerted conformation change of the three binding sites.

of F_1—one free, one loaded with ADP, and one with the ATP analogue AMP-PNP—have different conformations. Paul Boyer and John Walker were awarded the Nobel Prize (1997) for these results. However, the details of this mechanism are still a matter of debate.

Further investigations showed that the central γ-subunit rotates. The γ- and ε-subunits of F_1 together with the 12 c-subunits of F_o (shown in red in Fig. 4.7) form a rotor. This rotor rotates in a stator consisting of subunits-$(\alpha\beta)_3$, δ, a, b, b′, by which the conformations of each of the catalytic centers shown in Fig. 4.11 is changed. This model suggests that three molecules of ATP are formed by one complete revolution.

This model was confirmed by a startling experiment carried out by Masasuka Yoshida and Kazohiko Kinosito in Japan. These researchers attached a fluorescent molecule to the upper end of a γ-subunit contained in an F_o particle present in a membrane. Using a special video microscopy documentation it was possible to make visible that upon the hydrolysis of ATP the γ-subunit did in fact rotate. The F_o part functions as a type of nano motor. The velocity of rotation of the F-ATP-synthase in chloroplasts has been estimated to be up to 160 revolutions per second. To explain how this nano motor is driven by a proton gradient on the basis of known structural data, Wolfgang Junge (Osnabrueck, Germany) developed the model shown in Figure 4.12. In this model the a-subunit of the stator (shown in gray) possesses a channel through which protons can move from the outside of the membrane to a binding site of a c-subunit of the rotor, possibly a glutamate residue. At another site of the stator is a second channel through which the protons bound to the c-subunit can be released to the inside. By Brownian movement this proton-loaded c-subunit can rotate to the other proton channel where the proton is released, facilitating a proton transport driven by the proton gradient from the outside to the inside. But why does the rotation caused by Brownian movement proceed only in one direction? Two factors could be responsible for this: the spacial separation of the two channels and a positively charged arginine residue of the a-subunit of the stator. It is assumed that the positive charge of the arginine repels

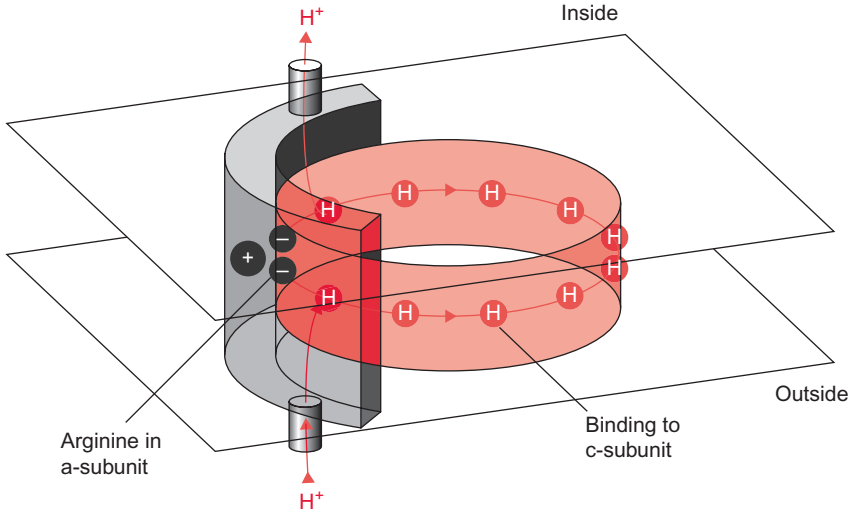


Figure 4.12 Model for the proton driven rotation of the rotor of the F_o part of the ATP synthase consisting of c-subunits. (After Junge et al., 1997.) The mechanism is described in the text.

the proton-loaded subunit and thus prevents a backward movement of the rotor, orientating the Brownian movement into one direction. Driven by a proton gradient that causes the loading and unloading of the proton binding sites at the respective channels, according to this model the nano motor rotates step by step like a ratchet in only one direction. In this way one full revolution causes the conformational change in the F_1-part leading to the formation of three molecules of ATP. Although an experimental verification of this model remains to be done, it gives an idea of how the nano motor of the ATP synthase may be driven by a proton gradient.

As discussed previously, several bacteria contain an **F-ATP synthase** that is driven by an Na^+ gradient. The model of the proton driven rotor allows the assumption that the subunit c of the Na^+ F-ATP synthase binds Na^+ ions and the two partial ion channels conduct Na^+.

It is still unclear how many c-subunits make up the rotor. Investigations of the number of c-subunits per F-ATP synthase molecule yielded values of 12 to 14 (chloroplasts), 10 (yeast mitochondria), and 12 (*E. coli*). Apparently in various organisms the number of c-subunits in the F_o part vary, therefore the number of protons required for one revolution to form three molecules of ATP will vary accordingly.

In photosynthetic electron transport the stoichiometry between the formation of NADPH and ATP is still a matter of debate

According to the model discussed here, chloroplasts with 14 c-subunits per rotor would require 14 protons for a complete rotation. Since three ATP molecules are formed during one rotation, this would correspond to an H^+/ATP

ratio of 4.7. Independent measurements indicated that in chloroplasts at least four protons are necessary for the synthesis of one ATP, which would be similar to the proton stoichiometry calculated for the rotor model. It is still not clear to what extent the Q-cycle plays a role in proton transport. In the linear (noncyclic) electron transport, for each NADPH formed without a Q-cycle, four protons (PS II: $2H^+$, Cyt-b_6/f complex: $2H^+$), and with a Q-cycle (Cyt-b_6/f complex: $4H^+$) six protons, are transported into the lumen (section 3.7). With a H^+/ATP ratio of 4.7, for each NADPH produced 1.3 ATP would be generated with the Q-cycle in operation and just 0.9 ATP without a Q-cycle. If these stoichiometries are correct, noncyclic photophosphorylation would not be sufficient to meet the demands of CO_2 assimilation in the Calvin cycle (ATP/NADPH = 1.5, see Chapter 6) and therefore cyclic photophosphorylation (section 3.8) would be required as well. The question concerning the stoichiometry of photophosphorylation is still not finally answered.

H^+-ATP synthase of chloroplasts is regulated by light

H^+-ATP synthase catalyzes a reaction that is in principle reversible. In chloroplasts, a pH gradient across the thylakoid membrane is generated only during illumination. In darkness, therefore, due to the reversibility of ATP synthesis, one would expect that the ATP synthase then operates in the opposite direction by transporting protons into the thylakoid lumen at the expense of ATP. In order to avoid such a costly reversion, chloroplast ATP synthase is subject to **strict regulation**. This is achieved in two ways. If the pH gradient across the thylakoid membrane decreases below a threshold value, the catalytic sites of the β-subunits are instantaneously switched off, and they are switched on again when the pH gradient is restored upon illumination. The mechanism of this is not yet understood. Furthermore, chloroplast ATP synthase is regulated by **thiol modulation**. By this process, described in detail in section 6.6, a disulfide bond in the γ-subunit of F_1 is reduced in the light by **ferredoxin** to form two -SH groups. This is mediated by reduced **thioredoxin (**section 6.6). The reduction of the γ-subunit causes the activation of the catalytic centers in the β-subunits. In this way illumination switches F-ATP synthase on. Upon darkening, the two -SH groups are oxidized by oxygen from air to form a disulfide, and as a result of this, the catalytic centers in the β-subunits are switched off. The simultaneous action of the two regulatory mechanisms allows an efficient control of ATP synthase in chloroplasts.

V-ATPase is related to the F-ATP synthase

Vacuoles contain a proton which transports V-ATPase and is conserved in all eukaryotes. Some V-ATPases transport Na^+ ions instead of protons. In

plants, V-ATPases are located not only in vacuoles (V = vacuoles), but also in plasma membranes and membranes of the endoplasmic reticulum and the Golgi apparatus. Genes for at least 12 different subunits have been identified in *Arabidopsis thaliana*. Major functions of this pump are to acidify the vacuole and to generate proton gradients across membranes for driving the transport of ions. V-ATPases also play a role in stomatal closure of guard cells. They resemble the F-ATP synthase in its basic structure, but are more complex. They consist of several proteins embedded in the membrane, similar to the F_o part of the F-ATPase, to which a spherical part (e.g., F_1) is attached by a stalk that protrudes into the cytosol. The spherical part consists of 3A- and 3B-subunits, which are arranged alternately like the ($\alpha\beta$)-subunits of F-ATP synthase. F-ATP synthase and V-ATPase are derived from a common ancestor. The exact number of protons transported per ATP depends on how many c-subunits the rotor of the F_o part contains. The V-ATPase is able to generate titratable proton concentrations of up to 1.4 mol/L within the vacuoles (section 8.5).

Vacuolar membranes also contain an **H^+-pyrophosphatase**, which upon the hydrolysis of one molecule of pyrophosphate to phosphate pumps one proton into the vacuole, but it does not reach such high proton gradients as the V-ATPase. H^+-pyrophosphatase probably consists of only a single protein with 16 transmembrane helices. It remains to be elucidated why there are two enzymes transporting H^+ across the vacuolar membrane. Plasma membranes contain a proton transporting **P-ATPase**, which will be discussed in section 8.2.

Further reading

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