Adenosine Triphosphate Synthesis Using an Electrochemically-driven Proton Pump

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By coupling the proton-release from a hydrogen-loaded palladium electrode, upon which was adsorbed an ATPase, the amount of ATP in solution is increased one hundred fold, as detected by the release of light from luciferin-luciferase.

The chemiosmotic hypothesis predicts that an artificially generated proton electrochemical potential difference should be able to cause the net synthesis of ATP in any energy-transducing membrane with a functional ATP synthase.[1] The first demonstration that this was so came from studies of thylakoids:[2] it was found that thylakoids equilibrated in the dark at acid pH could be induced to synthesize ATP when the external pH was suddenly increased from four to eight, creating a transitory pH gradient of four units across the membrane. Subsequently it was shown[3] that submitochondrial particles, subjected to an artificially imposed electrochemical proton gradient consisting of a pH gradient and membrane potential, catalysed the net synthesis of 2.5 nmol of ATP per mg of protein from ADP and phosphate ATP synthesis continued for about 6 s and was sensitive to uncouplers or oligomycin but insensitive to inhibitors of electron transport. These results demonstrated that an electrochemical gradient of protons can drive the synthesis of ATP by reversal of the proton-translocating ATPase independent of electron transport. In 1975 ATP synthesis was coupled with the action of membrane protonic pumps at an octane–water interface.[4] It was shown that the presence of a transmembrane potential is not a necessary condition for ATP synthesis by ATPase and the energy of proton solvation can be used for ATP synthesis.

Direct electrochemical ATP synthesis on an electrode surface has not been reported though a theoretical outline has been given.[5] It was shown, by thermodynamic analysis, that it is possible to envisage the synthesis of ATP by participation of a proton donor with an effective pK substantially lower than seven.

We report herein the direct electrochemical ATP synthesis on an electrode surface by use of an electrochemical proton pump. ATP was detected by measuring the light produced when it interacted with a luciferin–luciferinase couple (LLC). The light was measured with a cooled photomultiplier† and a picoammeter‡ to record the photocurrent.

ATP synthesis has been realized on a palladium electrode (2×10^{-5} m^2), which was modified by ATPase (obtained from Sigma). An activated and hydrogen-loaded Pd electrode was placed in solution of ATPase in autoclaved water (1 unit of ATPase per 0.05 ml water). (The Pd electrode was activated by cycling in Na_2SO_4 (20 mmol dm^{-3}) between −1.3 and +0.8 V *vs.* SCE and then dihydrogen was loaded into palladium potentiostatically at −1.0 V). The activated Pd electrode was then placed in an electrochemical cell with 10^{-5} mol dm^{-3} ADP + 10^{-11} mol dm^{-3} ATP in ATPase media[6] without initial degassing: it had a formal potential of −0.5 V *vs.* SCE. When the electrode was polarized at +0.2 V, light was emitted (which was produced as a result of the reaction between ATP and luciferin–luciferase couple) and this increased for 2 min. After disconnection, the Pd electrode had a potential *ca.* −0.3 V and the second attempt to generate ATP on the same electrode surface was unsuccessful. To improve the response and to increase the amount of electrochemically generated ATP, the concentration of ADP was increased to 5×10^{-4} mol dm^{-3}. Moreover, because a hydrogen-loaded Pd electrode surface, ADP[7] and ATPase[8] are all negatively charged (the isoelectric point of ATPase is between pH 4 and 5.5), $MgCl_2$ (5 mmol dm^{-3}) was added to the ATPase solution. As a result, the quantity of electrochemically generated ATP increased markedly and attained a concentration of 10^{-9} mol dm^{-3} (Fig. 1). ATP synthesis under an anodic polarisation lasted for more than 2.5 min and stopped after the electrode was disconnected. ATP generation on the same modified electrode was again successful: the shape of the curve was again produced only with a smaller amplitude. Thus, enzyme molecules are retained on the electrode surface after anodic polarisation and can be used again until complete exhaustion of all absorbed hydrogen. In accordance with the chemiosmotic theory,[1,4,5,9–11] it is necessary to have a proton stream through P-type-ATPases for ATP synthesis. It can be a pH difference drive stream[2,3] or transmembrane protons,[4] but in any case, protons have to go through the F_1 and F_2 subunits of ATPase to generate ATP. In this work, palladium electrodes have been used as a source of protons for ATP synthesis. The question arises as to the nature of the pH gradient generated at a Pd electrode. For the measurement of an H-loaded Pd electrode pH gradient, the electrode was placed in 20 mmol dm^{-3} Na_2SO_4 with pH 6.7 (E = 0.31 V) and was polarized at −1.0 V for 5 min. After polarization, the electrode was rinsed with deionised water and placed in a new

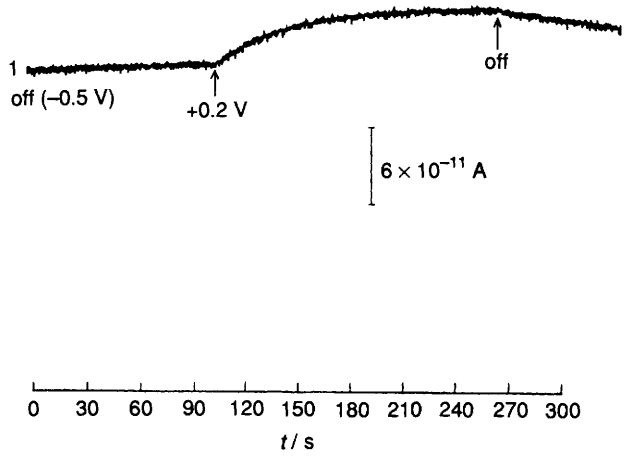

Fig. 1 Photocurrent changes during the electrochemical ATP generation (5×10^{-4} mol dm^{-3} ADP + 5×10^{-11} mol dm^{-3} ATP + 0.1 ml luciferin–luciferase cocktail, photomultiplier voltage 775 V)

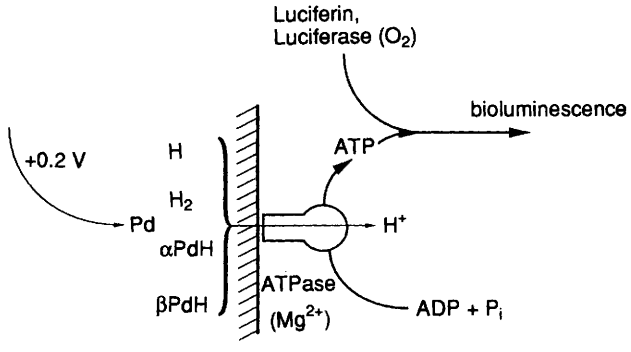

Fig. 2 Scheme illustrating how ATPase generates ATP and photocurrent on the surface of a H-loaded Pd electrode

portion of the same solution. After potentiostatic electrolyses at −1.0 V, rinsing and placing in a fresh 20 mmol dm^{-3} Na_2SO_4 solution, the H-loaded Pd electrode was polarized at 0.2 V for 3 min. As a result, a new formal electrode potential was established at −0.19 V and this potential difference corresponded to *ca.* 4 units of pH near the electrode surface and *ca.* 0.52 pH in the bulk of the solution. Therefore, the hydrogen-loaded Pd electrode can be used as a well-controlled proton pump in which a quantity of H^+ and the rate of proton generation can be regulated electrochemically.

We have demonstrated the direct electrochemical synthesis of ATP from ADP and phosphate ion on ATPase-modified electrodes without natural or artificially prepared ATPase-included membranes, membrane potentials and trans-membrane pH gradients. In this case, ATP synthesis is realised by a localised stream of protons from a Pd electrode to enzyme molecules adsorbed on the electrode surface (Fig. 2). It should make it possible to create a well-controlled ATP synthesis process which can be electrochemically regulated. The relevance of these observations to the synthesis of ATP in biology is, of course, uncertain but it establishes[4] that the essence of the process does not require a membrane.

Received, 8th December 1993; Com. 3/07274F

Footnotes

† Hamamatsu Type 7102 with an S1 cathode.
‡ Keithley Instruments 602 battery driven electrometer.

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