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Adenosine 5′-Triphosphate Synthesis in *Escherichia coli* Submitted to a Microsecond Electric Pulse†

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Received February 22, 1985; Revised Manuscript Received August 28, 1985

ABSTRACT: The total cytoplasmic ATP content (bound and free) increased in *Escherichia coli* when the bacteria were submitted to electric pulses with field strengths of 1–6 kV/cm and a decay time of 7–20 μs. The electron-transport chain was blocked by cyanide, and ATP synthesis was detected by a luminescence assay. The amount of newly formed ATP depends on the field strength. A total of 150 pmol of ATP was formed per milligram of bacteria submitted to a 3 kV/cm pulse. Synthesis was blocked by uncouplers and ionophores (valinomycin). The F_1F_0-ATP synthase inhibitor dicyclohexylcarbodiimide blocked a large part of this synthesis. Synthesis was not induced in unc mutants (unc B, unc D). The synthesis of ATP is related to the induced transmembrane potential, not to the Joule heating. A minimum 35–50-mV increase in membrane potential must be maintained for at least 12 μs to trigger this synthesis. This very fast energy transduction in bacteria is in good agreement with our previous results concerning submitochondrial particles. Because of the localized character of the induced membrane potential, these results are in agreement with the recent hypothesis of "mosaic proton coupling".

Conversion of the energy formed during respiration into the terminal phosphate bond of ATP[1] has been the focus of numerous studies using chloroplasts, mitochondria, or bacteria as experimental systems (Boyer et al., 1977). A striking similarity exists among all these systems not only in the enzymes involved (F_1F_0-ATP synthase) but in the mechanism of such a conversion (Boyer et al., 1977). The basic mechanism of energy transduction is postulated to be the conversion of the electrochemical gradient evoked by the respiratory chain into a chemical bond.

This hypothesis has been tested by the artificial creation of such a potential. If bacteria were first loaded with K^+ ions and then resuspended in a K^+-free medium, ATP synthesis was triggered in *Escherichia coli* and *Streptococcus lactis* by the addition of a small aliquot of the K^+ ionophore valinomycin (Maloney et al., 1974; Grinius et al., 1975). A complete analysis of this process in *E. coli* (Wilson et al., 1975) showed that a membrane potential was enough to induce the synthesis (no pH gradient was required). Furthermore, this last study (Wilson et al., 1975) demonstrated that the F_1F_0-ATP synthetase was involved in this conversion. Mutants where this enzyme was either lacking or inactive (unc) were unable to generate ATP when submitted to such a concentration pulse.

In these experiments, the ionic content of the cytoplasm was completely different from physiological conditions, and the consequences of this drastic change were unknown. Furthermore, the membrane structure was clearly affected by the ionophore. Recently, a direct electric alteration of membrane potential under less perturbing conditions has been developed (Kinosita & Tsong, 1977a; Teissié & Tsong, 1981a; Teissié et al., 1981). When a cell suspension is submitted to an electric field, a transmembrane potential is evoked. This potential is directly related to the external stimulus. Such a technique has been used in the study of ATP synthesis in chloroplasts (Witt et al., 1976), reconstituted bacterial systems (Rogner et al., 1979), and submitochondrial particles (Teissié et al., 1981; Knox & Tsong, 1984). Ionic transport against a concentration gradient can also be driven by such a stimulation (Teissié & Tsong, 1981b).

This paper shows that an external electric field may induce ATP synthesis in *E. coli*. This effect is associated with the induced membrane potential and not with a temperature increase. The study used electric pulses of various durations in the microsecond range. It provides evidence that energy transduction in the bacterial membrane is a very rapid process (several microseconds), which is triggered as soon as the membrane potential passes a critical threshold. Furthermore, this transduction is associated with a localized effect of the membrane potential in agreement with the "mosaic proton coupling" hypothesis (Westerhoff et al., 1984).

Materials and Methods

E. coli strains CB 0129 and AN 120 (unc A) were provided by Prof. Louarn (this institute). Mutants AN 719 (unc B)

† A preliminary report of this work was presented at the 36th meeting of the Société de Chimie Physique in Paris, 1982.

[1] Abbreviations: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N′*-dicyclohexylcarbodiimide; DNP, 2,4-dinitrophenol; EDTA, ethylenediaminetetraacetic acid; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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and AN 817 (unc D) were obtained from Dr Bachman (Yale University). Cells were grown in mineral medium M9 where glucose is the carbon source supplemented with thymine (29 μg/mL) and leucine (20 μg/mL). Supplements were added when needed. Bacteria were grown in batches of 30 mL at 37 °C with continuous air bubbling. Growth was monitored turbidimetrically with a Beckman spectrophotometer. It was assumed that 1 mL of a suspension with an absorbance of 1 at 640 nm contained 220 μg (dry weight) of cells. Cells were kept for 2 h in the stationary phase as checked by turbidimetry before harvesting.

Chemicals. *N,N'*-Dicyclohexylcarbodiimide (DCCD) was purchased from Fluka (Switzerland). Valinomycin, adenosine 5'-triphosphate (ATP), carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP), carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), dinitrophenol (DNP), and the luciferin/luciferase reactant were all obtained from Sigma. Inhibitors and ionophores were dissolved in 95% ethanol (final concentration of ethanol in the cell suspension was always less than 1%).

Depletion of Cytoplasmic ATP. The method was that described by Grinius et al. (1975). Cells in the stationary phase were first centrifuged (Sorvall RC5B centrifuge; Sorvall HB4 rotor; 5000 rpm during 10 min; 4 °C). The pellet was washed with 20 mL of 100 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) ($pH_{20°C}$ 8) and centrifuged again under the same conditions as above. The pellet was then resuspended in 20 mL of 250 mM sucrose, 15 mM NaCN, and 10 mM Tris-HCl ($pH_{20°C}$ 8). The cell suspension was then incubated for 2 h at 37 °C. The bacteria were then diluted to a suitable concentration.

Voltage-Pulse Experiments. Pulses were applied by means of a temperature-jump apparatus built in our laboratory (Teissié, 1979), where the discharge chamber was obtained from Messanlagen (FRG). The high voltage (up to 6 kV) stored in the capacitor (10, 20, and 50 nF) was discharged in the cell suspension. The discharge compartment was filled with 2.5 mL of the solution, but in fact, due to the large dead volume under the lower electrode only the upper 1.5 mL of the solution was effectively between the electrodes. It is well-known that the electric field decays with first-order kinetics. The half-lifetime of this process was measured by use of a dye indicator (phenol red) or by use of the turbidity change of the suspension. Both methods gave the same results. The temperature of the suspension was initially at 20 ± 1 °C. Turbidity changes were detected by a PM tube (EMI 9781) (England) and, after electronic amplification, stored in a Datalab DL905 transient recorder (England). They were then plotted on a chart recorder, and manual deconvolution was done.

ATP Content Determination. ATP was measured by bioluminescence with the method of Cole et al. (1967) with slight modifications. The 1.5-mL upper volume of the pulsed cell suspension was treated with 0.25 mL of 30% perchloric acid at 0 °C. After at least 15 min, the acid extract was neutralized with 0.75 mL of 1 M KOH. The luciferin/luciferase reactant was made fresh just before the measurement at a 10 mg/mL concentration. For the assay, 0.2 mL of 0.2 M glycylglycine (pH 7.4) was mixed with 0.7 mL of water in a Beckman glass scintillation vial. This mixture was cooled for at least 1 h in an Intertechnique SL 4000 scintillation counter (France). A 0.02-mL sample of the bacterial extract was then pipetted in this reaction mixture. Then, the luciferin/luciferase reactant (0.04–0.075 mL) was mixed. Counting was operated with full-window setting, without chemioluminescence subtraction during repetitive 6-s periods. Calibration was obtained by use of samples of a known ATP concentration that had been treated as the cell suspension (i.e., by perchloric acid followed by a KOH neutralization); with no added ATP, about 50 cpm was detected (electronic background); 100 000 cpm was observed when 20 pmol of nucleotide was added.

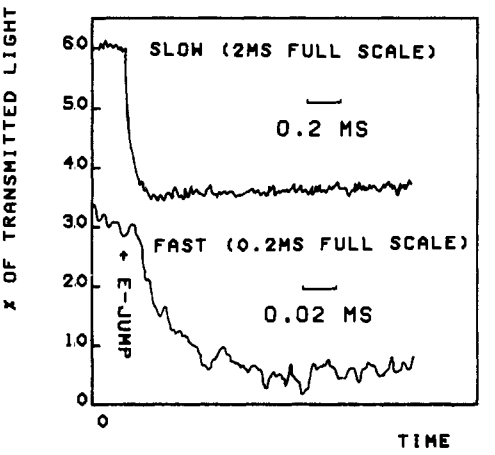

FIGURE 1: Turbidity changes associated with the electric pulsation of a bacteria suspension. *E. coli* (0.6 mg/mL) suspended in 0.25 M sucrose, 15 mM NaCN, and 10 mM Tris-HCl (pH 8) were pulsed ($E_{initial}$ = 5 kV/cm, C = 50 nF). The transmitted light was recorded at 575 nm. The 100% transmission was for the unpulsed sample. The electronic time constant was 1 μs.

In all our experiments, a reference sample containing the unpulsed bacterial suspension in the same buffer as the pulsed ones was counted first (less than 15 000 cpm). Its ATP content (basal level) was then subtracted from the level found in the pulsed samples in order to get the amount of newly formed ATP (synthesis). Within a given batch of cells, synthesis was reproducible with less than 10% fluctuation (under identical pulse conditions).

RESULTS

Spectroscopic Changes Associated with the Electric Pulse. The bacterial suspension is a turbid medium. Its turbidity was observed to change in a biphasic way when the pulse was applied. A fast decrease (during the first 500 μs) followed by a much slower increase was recorded (Figure 1). The magnitude of the first step is linearly related both to the strength of the initial field and to the decay time of the stimulus (data not shown). The dependence of the amplitude of the absorbance jump on the decay time of the field was the same whatever the method used to alter its value (modifying the ionic content of the pulsing media or changing the capacitance). The F_1F_0-ATP synthase inhibitor DCCD and the uncoupler FCCP were not observed to affect the spectroscopic dynamic signal.

ATP Synthesis by Electric-Pulsed E. coli Bacteria. When starved *E. coli* suspended in a buffer containing 15 mM NaCN (to inhibit the respiratory chain) were submitted to an electric pulse with an exponential decay, their cytoplasmic ATP content was observed to increase. Under our experimental conditions (applied voltage less than 6 kV), the temperature increase was less than 0.1 °C. Cells were viable after this treatment. In a control experiment cells that had been pulsed 5 times (E_0 = 5 kV/cm, C = 50 nF) were diluted back into a culture medium (M9). They were observed to grow as would untreated bacteria.

For a given batch of *E. coli* cells, the amount of ATP synthesized per milligram of bacteria was constant no matter what number of cells were present in the pulsation cell. From

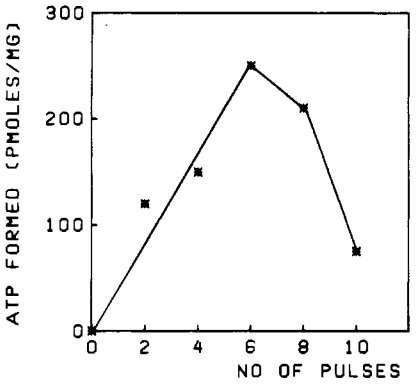

FIGURE 2: Newly formed ATP as a function of the number of successive pulses. *E. coli* (0.6 mg/mL) suspended in 0.25 M sucrose, 15 mM NaCN, and 10 mM Tris-HCl (pH 8) were submitted to repetitive pulses ($E_{initial}$ = 5 kV/cm, C = 50 nF). The delay between the pulses was 15 s (time needed to load the capacitance).

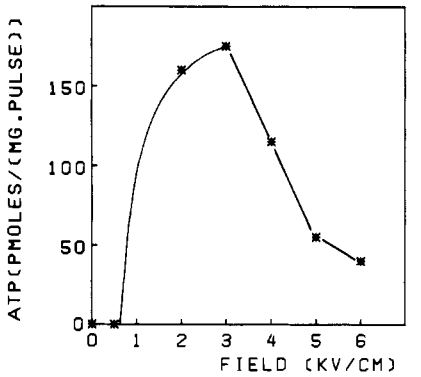

FIGURE 3: Influence of the strength of the electric field on the amount of newly formed ATP. *E. coli* (0.6 mg/mL) suspended in 0.25 M sucrose, 15 mM NaCN, and 10 mM Tris-HCl (pH 8) were submitted to two repetitive pulses (delay between each pulse 15 s, C = 50 nF). The indicated field strength is the initial value.

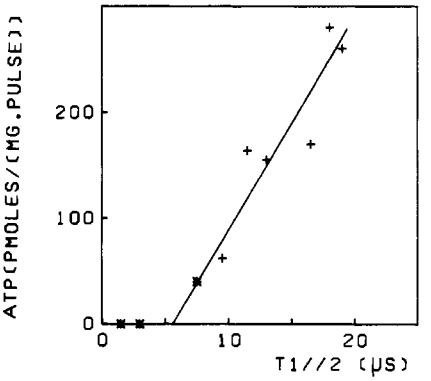

FIGURE 4: Influence of the half-time of the electric field decay on the amount of newly formed ATP. (+) *E. coli* (0.6 mg/mL) were suspended in a buffer (0.25 M sucrose, 10 mM Tris-HCl, pH 8) containing increasing amounts of NaCN (1.25–15 mM). The half-lifetime was obtained from the turbidity change experiments (see Figure 4). Five repetitive pulses ($E_{initial}$ = 5 kV/cm, C = 50 nF, delay between pulses = 15 s) were applied. (*) *E. coli* (0.6 mg/mL) suspended in 0.25 M sucrose, 15 mM NaCN, and 10 mM Tris-HCl (pH 8) were submitted to five repetitive pulses ($E_{initial}$ = 5 kV/cm, delay between pulses = 15 s). The loading capacitance was changed for each experiment, inducing a change in the associated half-lifetime (three capacitors were available; C = 10, 20, and 50 nF). This last parameter was monitored by the turbidity change.

one set of experiments to the other (i.e., for different batches of cells), the synthesis was reproducible within ±20% for identical pulsing conditions. The yield per pulse was 150 pmol of ATP synthesized per milligram of dry weight of bacteria (E_0 = 3 kV/cm, C = 50 nF, 15 mM NaCN). The starvation procedure did not appear to affect this yield because no direct correlation between this yield and the initial level of cytoplasmic ATP was observed.

The amount of synthesized ATP was a function of the number of pulses (Figure 2). The increase was found to be linear for up to six pulses (E_0 = 5 kV/cm, C = 50 nF, 15 mM NaCN, 15-s delay time between pulses). If pulsing were continued beyond this point, a decrease was observed. It should be mentioned that even with repetitive pulses the temperature change is always negligible. With 10 pulses (E_0 = 5 kV/cm), the calculated temperature change would be 1 °C if one neglected thermal dissipation. But, in fact, such a phenomenon occurs during the delay between each pulse; thus, the temperature increase is much less than 1 °C. This decrease after repeated pulses was linked to the number of pulses not to the strength of the applied field. The same effect was indeed observed with E_0 = 3 kV/cm (keeping unchanged the other experimental conditions).

Dependence of ATP Synthesis on Field Strength. The synthesis was observed to be under strict control of the magnitude of the applied field. For low intensities of the initial field (less than 1 kV/cm, C = 50 nF, 15 mM NaCN), no increase in the cytoplasmic ATP content was detected; for values between 1 and 3 kV/cm (same conditions), the increase is rather dramatic up to a stationary level close to 150 pmol/mg of dry weight per pulse. This level is less for higher initial values of the field (same conditions). A tentative explanation of these observations is given under Discussion (Figure 3). It should be noticed that under our experimental conditions the temperature increase is always small (less than 1 °C).

Dependence on Decay Time of External Field. With our experimental set-up, for a given initial field strength, the decay time may be changed by two methods. The decay time is equal to $T = RC$, where C is the value of the capacitance whose discharge induces the field and R is the equivalent resistance of the solution between the electrodes where the discharge occurs. Thus, by changing the ionic strength of the pulsing medium (for example, by decreasing the concentration of NaCN), R would be changed. As shown in Figure 4, when NaCN is decreased from 15 to 1.25 mM stepwise, the amount of newly formed ATP is increased progressively 4 times. Under these conditions, the temperature change stayed constant (same amount of dissipated energy), but of course, the composition of the pulsing media was slightly changed. Nevertheless, it should be noticed that the concentration of NaCN is always high enough to inhibit the respiratory chain.

The decay time of the applied field is linearly related to the value of the discharge capacitance when the ionic content of the pulsing medium is kept the same. As shown in Figure 4, when the high ionic content pulsing buffer (15 mM NaCN) was used, but smaller values of the capacitance (10 and 20 nF) (3 and 1.5 μs as decay times), no synthesis is detected under the same pulsing conditions (5 times, 5 kV/cm).

An interesting observation of these two sets of experiments is that the data did not seem to extrapolate to the origin, which suggests that no synthesis would be obtained for a decay time that is short but different from zero. This observation will be discussed below.

Effects of Inhibitors and Ionophores. In an attempt to localize the site of this ATP synthesis, the effect of several specific inhibitors of the bacterial F_1F_0-ATP synthetase was examined. DCCD is known to inhibit ATP synthesis (Fillingame, 1980). As shown in Table I, the electric field induced ATP synthesis was strongly reduced when DCCD was present.

Table I: Effects of Inhibitors, Uncouplers, and Ionophores on the Amount of Newly Formed ATP in Electric-Pulsed Bacteria[a]

conditions	ATP formed [pmol [mg of bacteria (dry weight)]$^{-1}$ pulse^{-1}]	% (untreated as reference)
E. coli	50	100
+1.5 mM DCCD	28	56
+50 μM valinomycin	33	67
E. coli treated with EDTA	10	100
+1.5 mM DCCD	5	50
+50 μM valinomycin	0	0
E. coli	100	100
+20 μM FCCP	13	13
+20 μM CCCP	15	15
+200 μM DNP	0	0

[a] Bacteria (0.6 mg/mL) in 0.25 M sucrose, 15 mM NaCN, and 10 mM Tris-HCl (pH 8) were pulsed 5 times (delay between pulses = 15 s) ($E_{initial}$ = 5 kV/cm, C = 50 nF). All additives were in ethanolic solution. ATP basal level was 35 pmol/mg of bacteria after the starvation process.

Table II: Electric Field Effects on unc Mutants[a]

strain	mutation	altered subunit	synthesis: pmol mg^{-1} pulse^{-1}	synthesis: %
CB 0129	wild	none	150	100
AN 120	unc A	α in F_1	150	100
AN 817	unc D	β in F_1	none	0
AN 719	unc B	B in F_0	none	0

[a] The synthesis is the increase of cytoplasmic ATP content induced by the electric pulses (5 time, 3 kV/cm, 15 mM NaCN). Results obtained with the wild strain were taken as 100%.

This inhibition was not changed after permeabilization of the outer membrane by treating the bacteria with EDTA before starvation (50 μM EDTA during 2 min at 37 °C). But it should be noticed that the yield of synthesis was reduced after the EDTA treatment. Proton conducting uncouplers (FCCP, CCCP, and DNP) significantly reduced or blocked the field-induced ATP synthesis (Table I).

Valinomycin is known to increase the permeability of the bacterial membrane to K^+ ions. But due to its high molecular weight, it cannot permeate across the outer membrane in untreated bacteria. Pretreatment of the cells with EDTA is needed in order to obtain the permeabilization of the plasma membrane by valinomycin. After such a treatment, valinomycin completely inhibits the field-induced synthesis (Table I).

Pulsation of unc Mutants. Genetic characterization of the unc operon in *E. coli* is now well documented [see Fillingame (1980) for a review]. Mutations affecting each subunit have been isolated. We have pulsed three different mutants the localization of the altered subunit and the nature of the mutation were defined, under the conditions described above. Results are shown in Table II. No synthesis at all is observed for unc B and unc D mutants. Surprisingly, the behavior of unc A is the same as that of the wild strain (same yield, same influence of the initial value of the field).

Electric Field Induced Pore Opening in Bacterial Membrane. The opening of reversible pores in the membrane as a consequence of the induction of the membrane potential is one of the best documented effects of electric fields on cell suspension [see, for example, Teissié & Tsong (1981b)]. We have checked if such an effect was present in our experiments by application of the pulses with the usual buffer supplemented with a low molecular weight radioactive tracer ([^{14}C]sorbitol) and measurement of the radioactivity that remained in the bacterial pellet after centrifugation and washing. The measured radioactivity kept the same level as the unpulsed sample as long as the initial value of the field is smaller than 3 kV/cm and increased strongly for stronger field intensities (Figure 5).

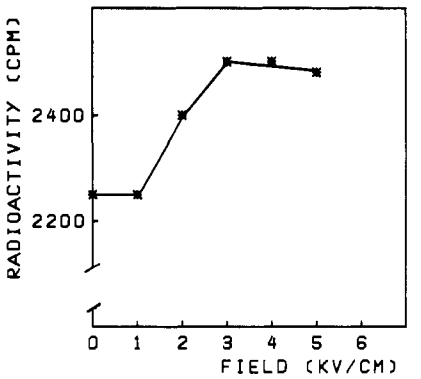

FIGURE 5: Pore opening in *E. coli* membranes. The bacteria were pulsed in a medium containing 200000 cpm of [^{14}C]sorbitol with fields of different initial intensities (C = 50 nF, 15 mM NaCN). After two pulses, the bacteria were washed several times, and the radioactivity remaining in the pellet was counted. The relatively high cpm background is linked to the tracer molecules, which are trapped in the intermembraneous space, and to the fact that as reported under Materials and Methods only 60% of the bacterial suspension is pulsed. The sharp increase obtained above 1 kV/cm is indicative of the opening of reversible pores in the membrane through which the radioactive tracer can penetrate into the cytoplasm.

Discussion

The above-reported experiments provide clear evidence that an externally applied electric field induces ATP synthesis in *E. coli* where the respiratory chain is completely inhibited by cyanide. The physical trigger of this synthesis was proved not to be the increase in temperature linked to the electric pulses (Joule heating). Evidence is provided as follows: (1) the temperature increase is always vary small (much less than 1.5 °C), (2) the synthesis is not directly correlated with the temperature increase (dependence on applied voltage and loading capacitance), and (3) for a given temperature increase the amount of newly formed ATP varies with the ionic strength. A direct effect of the electric field inducing an ionization of membrane functional groups and thereby acting as a trigger would be rapid (on the order of 10^{-14} s) (Schwarz & Bauer, 1974) and thus would not be affected by the decay time of the electric field as we observed by changing either the loading capacitance or the ionic strength of the pulsation buffer.

As recently reported (Kinosita & Tsong, 1977a; Teissié & Tsong, 1981a), the major effect of the electric pulsation of a cell suspension is not heating, but is the generation of a transmembrane potential. Some of our observations suggest that at least part of the synthesis occurs at the membraneous level and is directly related to its permeability (effect of uncouplers, of valinomycin). In fact, the induced potential would play a central role in this phenomenon. As reported elsewhere (Kinosita & Tsong, 1977a; Teissié & Tsong, 1981a), its magnitude is linearly related to the strength of the external field and to the size and the relative permeability of the pulsed vesicle. Its time dependence is close to the one of the applied field as shown by the theoretical approach (Kinosita & Tsong, 1977b).

In an attempt to determine the relationship between induced potential and synthesis activity, we assumed that all bacteria were oriented with their long axis parallel to the field lines. This orientation is suggested by the spectroscopic data. Bacteria are rods that when submitted to an electric field will

behave as dipoles and would be reoriented parallel to the field. This effect has already been observed with DNA molecules in similar experiments (Dourlent et al., 1974). A theoretical approach showed that such an orientation would have a time dependence close to the one of the applied field (O'Konsky & Haltner, 1957). Similar experiments performed on purple membrane fragments (Shinar et al., 1977) gave identical results: linear dependence of the light scattering change on the applied voltage and field-induced orientation time of the order of the pulse duration. The very fast turbidity change is the consequence of the field-induced orientation process, which is fast as shown in O'Konsky & Haltner (1957). The slow process is due to the randomization of the orientations associated with Brownian motion (no electric field was then applied on the sample). At the threshold value (Figure 3) of 1 kV/cm, the membrane potential increase may be calculated when one assumes that the conductivity of the *E. coli* membrane is negligible compared to those of the external buffer and of the cytoplasm. Using the calculations of Bernhardt & Pauly (1973) and assuming that the axial ratio for *E. coli* is 2:1:1 (the bacteria is considered as an ellongated ellipisoid: $2 \times 1 \times 1$ μm), we got a potential increase of 121 mV when bacteria are oriented with their long axis parallel to the field lines. If the bacteria were oriented in the crossed position, then the maximum value of the induced potential (i.e., at a position facing the electrodes) would be only 85 mV as derived from Bernhardt & Pauly (1973). This is less than the value calculated for a bacteria with the long axis parallel to the field lines (121 mV). As with this orientation, the induced potential always reached, for a given field, a higher value than for the other orientations, and the associated potential value (121 mV) should be considered as the threshold.

As reported for bacterial photophosphorylation (Melandri et al., 1980), ionophores would dissipate the induced potential increase by increasing the ionic permeability of the membrane. Another piece of information provided by our experiments is the time dependence of this electric pulse induced synthesis. The rise time of the induced potential is very short (about 0.1 μs) as can be calculated from the work of Kinosita & Tsong (1977b). As a consequence, it is not going to alter the value of the induced potential as a consequence of a delay between induction of the potential and decay of the field (Teissié & Tsong, 1981). Our results showed that if the half-lifetime of the field decay is too short (3 μs if the initial field strength is 5 kV/cm), no synthesis is observed (Figure 4). As shown in Figure 3, when the half-lifetime of the decay is 7.5 μs, the initial magnitude of the field required to induce the synthesis is 1 kV/cm. By comparing the decays of the fields in these two experiments, we see that the field in the second experiment becomes larger than that in the first 12 μs after the pulse trigger. We propose that the induced potential must be maintained above the threshold during at least 12 μs. The membrane potential threshold is then obtained from this last value and from the 1 kV/cm threshold (initial value, with a decay time of 7.5 μs). The threshold field is thus 0.3 kV/cm. Taking into account the relationship between field and induced potential (see above), the potential increase threshold is computed to be 36 mV. One should keep in mind that a resting potential is present in intact bacteria (about 100 mV); then, the total potential needed to induce the synthesis should be evaluated as about 150 mV (resting potential plus electric field induced increase). This set of values (150 mV, 12 μs) may be compared with the data obtained with those for chloroplasts (Witt et al., 1976) and submitochondrial particles (Teissié et al., 1981) for which a very close agreement is obtained. Such a conclusion, "the membrane potential must be increased from more than 40 mV during more than 12 μs to trigger ATP synthesis", appears as a general property of ATP synthase containing membranes (mitochondria and bacteria). The conclusion can be partly extended to the chloroplasts as far as the membrane potential is concerned; a great difference in the time required seems to be present. As far as reconstituted thermophilic bacterial systems are concerned (Rogner et al., 1979), no threshold data are available, but experiments were performed with pulsing times in the millisecond range and calculated membrane potential increases far above the threshold just given (200 mV as compared with 150 mV).

Trying to relate our threshold potential to the Gibbs energy change of ATP synthesis would be misleading. It is well-known that this thermodynamic parameter is under the dependence of the actual activities of all the compounds (nucleotides, cations, protons) involved in the synthesis. An accurate determination of these concentrations inside *E. coli* under our experimental conditions could not be done.

The precise identification of the enzymes involved in this induced synthesis is indirectly obtainable from our experiments. First of all, we may conclude without any doubt that they are located in the membrane and dependent on the membrane potential (effect of uncouplers and ionophores). Furthermore, the partial inhibition induced by DCCD suggests that the F_1F_0-ATP synthetase is directly involved in this process as was shown in the previous experiments on submitochondrial particles (Teissié et al., 1981). It is interesting to note that DCCD did not completely inhibit the electrically induced ATP synthesis in thermophilic bacteria reconstituted systems (Rogner et al., 1979). Direct evidence that the F_1F_0-ATP synthase is implicated in this synthesis is given by the experiments with the unc mutants. The electric field induced synthesis is abolished when the β subunit of F_1 or the B subunit of F_0 is affected. The results with AN 120 (mutation on the α subunit of F_1) are amazing, but one should keep in mind that this mutation affects the interaction between the α and β subunits, and we may suppose that this is bypassed by the process elicited by the electric field. From published data, a rough estimation of the number of ATP synthases involved in this process was computed as follows. Assuming that one DCCD molecule is bound to four C subunits of F_0 (M_r 8K) (Friedl et al., 1981) and that there are four to six C subunits per F_0 (Fillingame, 1981), one DCCD molecule is bound per F_1F_0-ATP synthase. Since 0.35 nmol of [^{14}C]DCCD is bound per milligram of *E. coli* protein (Fillingame, 1975) and the protein to dry bacteria ratio is 50% (w/w), 175 pmol of F_1F_0-ATP synthetase is present per milligram of *E. coli* (dry weight). From our results (up to 150 pmol of ATP is synthesized per pulse per milligram under optimum conditions), we conclude that four ATP molecules are synthesized for every five F_1F_0-ATP synthase molecules. With our electric field jump technique, the induced potential is position-dependent on the surface of the bacteria and only half of the membrane surface is polarized in the right direction for the activation of the F_1F_0-ATP synthase. It is therefore concluded that the synthesis of more than one ATP molecule per activated F_1F_0-ATP synthase is induced in our experiment as for SMPs (Knox & Tsong, 1984).

The rather complicated influence of the field strength on the synthesis in this bacterial system as compared with results obtained on mitochondrial preparations (Teissié et al., 1981; Hamamoto et al., 1982; Knox & Tsong, 1984) appears to be linked to the opening of pores in the bacterial membrane. When pores are opened, they electrically short-cut the mem-

brane potential (and disturb the structure of the membrane), thus removing the possibility for the membrane to synthesize ATP under the assumption of the delocalized chemiosmotic hypothesis. But from the comparison of the results given in Figures 3 and 5, we can see that pore opening is not linked to a complete suppression of the synthesis but only to its decrease. A computer simulation (which will be described in detail elsewhere) shows that the experimental results are in agreement with a model where the pore opening has only a small-range effect in abolishing the synthesis. Thus, our results are in agreement with a model where the membrane potential induced ATP synthesis is very localized as suggested in the mosaic protonic coupling hypothesis (Westerhoff et al., 1984).

As shown in the experiments where increasing numbers of pulses were applied to the bacterial suspension, this technique does not appear to damage the cells (at least up to six pulses), for the rate of synthesis remains constant (linear increase of formed ATP with number of pulses as shown in Figure 2). This is in agreement with other reports (Sale & Hamilton, 1968).

In a similar way, as we concluded for the experiments performed with submitochondrial particles (Teissié et al. 1981), the electric field induction of ATP synthesis gives direct evidence of the very fast transduction of an electrical potential into some form of energy stored in the membrane. Taking into account the rather long delay between the electric pulses and the detection of the increase in ATP content (several seconds), we cannot conclude at the present time that there is a direct conversion of the induced potential into a chemical bond.

This fast energy transduction appears as only one step in the complete "in vivo" respiratory process. As 960 ATP molecules are formed per minute per F_1F_0-ATP synthase under stationary conditions (Fillingame, 1981), one ATP molecule is formed every 60 ms, i.e., a "synthesis" time that is 10^4 longer than the one we observed. As at pH 6.5 (but with an unknown potential), 30 protons are extruded per second per F_0 in stripped vesicles (Fillingame, 1981); a proton is ejected every 30 mS. This last process is thus 5000 times slower than the induced energy transduction.

The present results provide direct experimental evidences of the occurrence of a very fast energy transduction in bacterial membranes. The strong analogy of the results with those we previously obtained with submitochondrial membranes (Teissié et al., 1981; Knox & Tsong, 1984) allows the extension of this conclusion to the mitochondrial membrane. An increase of the transmembrane potential up to 150 mV during at least 12 μs induces a transient state of the membrane, which results in the synthesis of ATP by the F_1F_0-ATP synthase. This effect is observed to be very localized as suggested by the mosaic coupling hypothesis (Westerhoff et al., 1984).

The different steps that may be involved in this process (conformational changes, proton transfer, or other) are under investigation by use of model systems (bacterial vesicles, reconstituted systems) with direct spectroscopic detection. A preferential conduction of protons along lipid/water interfaces has in fact been observed in our laboratory (Teissié et al., 1985; Prats et al., 1985).

ACKNOWLEDGMENTS

Thanks are due to Professors Pedersen and Tsong (Johns Hopkins University) for their comments on the manuscript, to Doctors Louarn and Fayet (Toulouse) for their helpful advice in the experiments, and to Professor Sulfridge (Towson State University) for her help in writing the manuscript.

Registry No. ATP, 56-65-5.

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