ENERGY-LINKED TRANSPORT OF PERMEANT IONS IN ESCHERICHIA COLI CELLS: EVIDENCE FOR MEMBRANE POTENTIAL GENERATION BY PROTON-PUMP

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SUMMARY. The fluxes of synthetic penetrating anions and cations in the energized E.coli cells in opposite directions have been demonstrated: anions moved out and cations moved into the cells. The energy-linked uptake of permeant cations was coupled with the outflow of H^+ ions from the cells. The uptake of permeant cations by deenergized cells was observed following non-enzymatic generation of a membrane potential, negative inside. The value of a membrane potential calculated from the distribution of permeant cations was close to I40 mV.

The problem of conversion of biomembrane-produced energy into electric form has been investigated (I-6) by means of synthetic ions penetrating phospholipid membranes. It has been concluded (I) that these ions of very different structure can be transferred across the biomembrane without being bound to a translocase adapted for the transfer of some natural ion. According to this point of view the difference of electrostatic potentials across the membrane is a driving force for the transport of ionic penetrants. Thus, one can judge from the direction of ion movement about the electric field orientation across the biomembrane.

Recently the energy-linked uptake of K^+ and permeant cation, dibenzyl dimethyl ammonium, by vesicles and intact cells of E.coli has been observed (7,8-I0).

The object of the present paper was to examine the role of proton-pump in the energy-linked transport of ionic penetrants in E.coli cells.

MATERIALS AND METHODS. The bacterial strain used was E.coli W945 (pro^-,lac^-,B_I^- , str-r), kindly provided by Dr.D.M.Goldfarb, Institute of General Genetics,Moscow,U.S.S.R. Stock culture was maintained on solid medium,containing cazein hydrolysate,yeast extract and agar. The liquid culture medium used was that of Fisher et al(II), with glucose as carbon source. An inoculum from the agar slope was grown for IO hours at 38°C under aerated conditions. The harvested cells were treated with Tris and EDTA,exactly as described by West and Mitchell (I2). The preparate of E.coli cells with partially depleted energy sources was used in some experiments (see Fig.I). The energy sources in

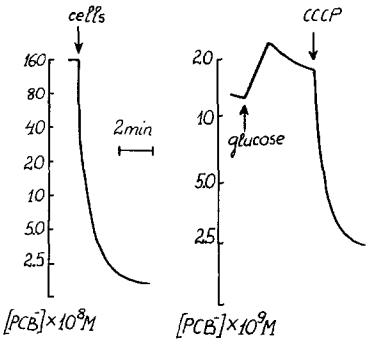

Figure I. Time-course of the PCB^- uptake by E.coli cells. Incubation medium contained: 0.I5M KCl,0.025M Tris-HCl(pH7.4) and $I.6 \cdot IO^{-6}$M PCB^-.Additions: arsenate-treated cells (5 mg dry wt./ml), $4 \cdot IO^{-3}$M glucose and $2 \cdot IO^{-6}$M CCCP.

E.coli were depleted by I hour incubation at room temperature under aerated conditions in the presence of IOmM sodium arsenate. The stock bacterial suspension in 0.25M sucrose was stored at 0°C. The accumulation of cationic penetrants in the E.coli cells was measured by a modified method of Harold and Papineau(5). Cells were incubated with cations for 5 minutes. Samples (5ml) of the cell suspension were taken from aerobic experimental vessel,

layered over 0.88M sucrose (3ml) and then pelleted at 2-4°C temperature. The uptake of cation was calculated from the optical density changes in the supernatant, as described by Harold and Papineau (5).

From the results of four parallel measurements the standard error was calculated. The effective quantities of H^+ and K^+ in the incubation medium were measured simultaneously by means of the ion-selective electrodes connected with sensitive electrometers and strip-chart recorders. The concentration of phenyldicarbaundecaborate (PCB^-) in the incubation medium was measured using phospholipid film, as described earlier (3,I3).

RESULTS AND DISCUSSION. Figure I demonstrates changes in the concentration of a permeant anion, PCB^-, induced by the addition of cells pretreated with arsenate and the subsequent energization of these cells. One can see that the addition of cells to the PCB^- solution resulted in a fast decrease of concentration of free PCB^-, the effect being due to very high distribution coefficient for PCB^- in the lipid-water system (I3). Energization of cells by the addition of glucose induces PCB^- efflux, thereby increasing the PCB^- concentration in the incubation mixture. A further decrease in the PCB^- concentration took place after deenergization of cells by the addition of uncoupler carbonylcyanide p-trichloromethoxyphenylhydrazone (CCCP). The PCB^- concentration stabilized at the level lower to that observed before glucose addition. It has been observed in control experiments that the cells treated with arsenate retained the ability to oxidize the endogenous substrates. This fact indicates that complete deenergization occurred only after the treatment with uncoupler. Thus, decrease of PCB^- concentration lower than the level observed before adding glucose seems to be

related with complete deenergization of cells by uncoupler.

The data characterizing the uptake of DDA^+ by E.coli cells are given in Table I. It is seen that the energized cells absorb DDA^+ from the incubation medium in the absence of PCB^-(Expt.I). In the presence of PCB^- the cells accumulate additional amount of DDA^+(Expt.2), the process being sensitive to uncoupler (Expt.3).

The concentration ratio calculated from the data of Table I corresponds to membrane potential of the order of - I40 mV.

Figure 2 shows the time-course of the effective quantities

Table I. Energy-linked uptake of DDA^+ by E.coli cells.

E.coli was suspended at a cell density of I.02 mg dry wt./ml in 0.25M sucrose with 3mM glycil-glycine (pH7.2) medium, incubated for 5 min under aerated conditions at room temperature. Than samples of the cell suspension were taken from the vessel and pelleted. Concentrations of DDA^+ were calculated from the data of four parallel measurements.

No.	Conditions of experiment	Uptake of DDA^+ by cells (nmoles/mg dry wt.)
I	With $5.90 \cdot 10^{-4}$M DDA^+. After the addition of cells the concentration of DDA^+ lowered to $3.25 \cdot 10^{-4}$M.	265 ± 62
2.	With $5.90 \cdot 10^{-4}$M DDA^+ and $1 \cdot 10^{-6}$M PCB^-. After the addition of cells the concentration of DDA^+ lowered to $1.74 \cdot 10^{-4}$M.	4I6 ± 20
3.	With $6.76 \cdot 10^{-4}$M DDA^+, $1 \cdot 10^{-6}$M PCB^- and $2 \cdot 10^{-5}$M CCCP. After the addition of cells the concentration of DDA^+ lowered to $3.66 \cdot 10^{-4}$M.	3I0 ± 24

of H^+ and K^+ ions entering the aerobic incubation medium with E.coli cells in response to the DDA^+ addition. Experiment A was carried out in the presence of PCB^- and experiment B in the

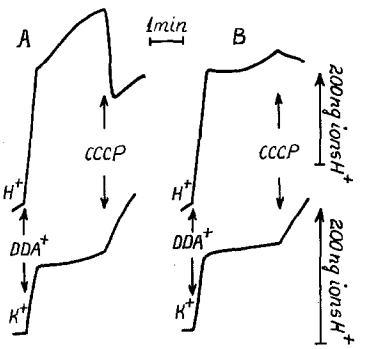

Figure 2. Time-course of K^+ and H^+ concentration changes in the incubation medium on adding DDA^+ and CCCP to aerobic suspensions of E.coli cells. Incubation medium contained: 0.25M sucrose, $3 \cdot 10^{-3}$M glycil-glycine, cells (2.2 mg dry wt./ml) and $1 \cdot 10^{-6}$M PCB^- (Expt.A). During aerobic incubation at room temperature for 7 min pH of suspension drifted from 7.2 to 7.0. Additions: $1 \cdot 10^{-3}$M DDA^+ and $8 \cdot 10^{-6}$M CCCP.

absence of this anion. Experiment A shows that H^+ ions enter (or OH^- ions leave) the medium in response to the DDA^+ addition. It also shows that during the effective entry of H^+ ions there is a small exit of K^+. It is seen that after the addition of uncoupler H^+ ions leave and K^+ ions enter the medium. In control experiments (not shown) a great stimulation of K^+ efflux by valinomycin has been observed. The experiment carried out in the absence of the cells showed that a rapid non-energy-linked response of K^+ ion--selective electrode was caused by the significant, but low, reactivity of the electrode to DDA^+. A control experiment showed that non-energy-linked pH response to DDA^+ addition was mainly caused by DDA^+ interaction with glycil-glycine.

Experiment B shows that in the absence of PCB^-, i.e. under the conditions where energy-linked uptake of DDA^+ is greatly diminshed (see Table I), the uncoupler-sensitive pH changes are practically abolished.

The stoicheiometry between the effective outflow of H^+

and the inflow of cationic penetrants has been examined. The premeant cations of triphenyl methyl phosphonium, $TPMP^+$, were used. It was not possible to measure both $TPMP^+$ and H^+ movements in the same suspension simultaneously because the withdrawal of portions of the suspension for $TPMP^+$ determinations disturbed the pH measuring system. Parallel sets of measurements were therefore done on cells from the same stock suspension. The effective quantities of H^+ entering and $TPMP^+$ leaving the incubation medium, calculated from the data of parallel measurements, showed that the stoicheiometric ratio of effective H^+ translocation/$TPMP^+$ translocation was close to I.

Since, as it is widely accepted, H^+ ions in the presence of uncoupler are considerably more mobile in the lipid membrane phase of cells than OH^- ions, it was possible to use the pulses of non-neutralized Tris-base to establish a trans-membrane diffusion potential of H^+ ions, negative inside. Evidence validating the influence of membrane potential on the concentration of permeant ions in the cells is shown in Fig.3. The experiment demonstrates the time-course of $TPMP^+$ quantities in the cells. At zero time the cells were added to the aerobic medium. At intervals indicated, samples were quickly taken from suspension, pelleted and supernatants were analyzed. It is seen that the uptake of $TPMP^+$ consists of two phases: an extensive uptake by energized cells and a less extensive uptake which was independent of energization.

Data of Fig.3 indicate that when the membrane is freely permeable to H^+ the pulse of non-neutralized Tris-base leads to the extensive uptake of $TPMP^+$ by deenergized E.coli cells. The experiment shows that the accumulated quantity of $TPMP^+$ drifts during 20 min to the initial level. All these results support the view that alkalization of incubation medium allows H^+ to diffuse outward down the

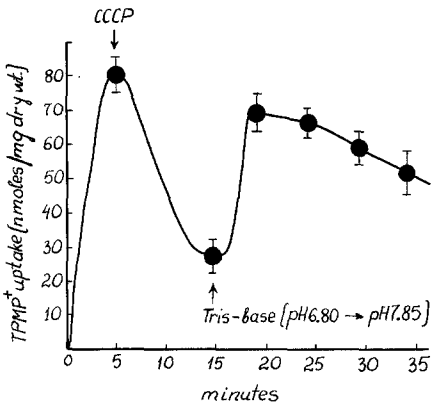

Figure 3. The uptake of $TPMP^+$ by the deenergized E.coli cells in response to a membrane potential. Details were as in Fig.2A except that the vessel contained 1.04 mg dry wt. of cells per ml. The cells were added at zero time. During aerobic incubation at room temperature for 15 min pH of suspension drifted from 7.2 to 6.8. After that the non-neutralized Tris-base was added and pH quickly shifted to 7.85. Additions: $1 \cdot 10^{-6}$M CCCP and solid Tris-base.

concentration gradient. The diffusion of H^+ ions generates a membrane potential which, in its turn, drives the uptake of cationic penetrant, $TPMP^+$.

The experiments reported in this paper have demonstrated that the fluxes of synthetic penetrating anions and cations in the energized E.coli cells have opposite direction: anions move out and cations move into the cells. The stimulating effect of lipophilic anions on the energy-linked uptake of cationic penetrants makes this process similar to the cation permeation through artificial membrane (13,3).

The accumulation of permeant cations caused the outflow of H^+ ions from the cells. Transmembrane protonophores-uncouplers of oxidative phosphorylation induced the inflow of H^+ ions. Sensitivity towards protonophores indicates that the energy-linked uptake of permeant cations leads to the appearance of a transmembrane pH gradient. These results are in favour of the chemiosmotic coupling conception (12,14) that in the energized state a proton-pump generates

a membrane potential,negative inside E.coli cells.

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