Ionic and Energetic Changes at Competence in the Naturally Transformable Bacterium *Streptococcus pneumoniae*

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Addition of competence factor extracts to trigger competence in a culture of *Streptococcus pneumoniae* induced an increase in the intracellular pH and the Na⁺ content of the bacteria without any change in the K⁺ pool or in the membrane potential. These ionic shifts were concomitant with a stimulation of glycolysis that resulted in an enhanced ATP pool. Thus, in transforming conditions, at extracellular pH 7.8, competent bacteria presented a particularly high energetic state resulting from an increase in Δp H and in the ATP pool, associated with an enhanced Na⁺ content. These features are discussed in the context of homeostasis regulation in response to an environmental stimulus.

INTRODUCTION

Competence for genetic transformation in *Streptococcus pneumoniae* is a transitory physiological state controlled by an exported activator 'competence factor', CF (Pakula & Walczak, 1963; Tomasz & Hotchkiss, 1964; Tomasz & Mosser, 1966; Leonard & Cole, 1972), whose target is likely to be on the surface of the bacteria, i.e. on the cell wall and/or on the cytoplasmic membrane (Ziegler & Tomasz, 1970; Kohoutova, 1973; Horne *et al.*, 1977).

During development of competence, a transient increase in the initial rate of isoleucine uptake, with an optimum at the peak of culture transformability is observed (Trombe, 1983). In our strains of *S. pneumoniae* a single transporter coupled to the protonmotive force (Δp) has been described for isoleucine and the maximum transport velocity, V , is modulated by the value of Δp (Trombe *et al.*, 1984). Therefore, variations of the initial rate of isoleucine uptake at competence are likely to reflect modulation of Δp resulting from modulation of ionic fluxes during competence development. Changes in ionic fluxes are observed in other pleiotropic responses to environmental conditions such as osmoregulation in *Escherichia coli*, which involves K⁺ regulation (Laimins *et al.*, 1981; Meury & Kepes, 1981). In the anaerobic bacterium *S. pneumoniae* ATP derived from glycolysis is the chief energy donor for the generation of ionic gradients via cation ATPases (Heefner & Harold, 1982; Kakinuma & Harold, 1985; Rosen, 1987). In this paper, the ATP content and the size of ion pools during competence development after stimulation by CF are described.

METHODS

Chemicals. Nutrients used for growth media were from Difco; other reagents were of analytical grade. Monensin was provided by Lilly Research, Surrey, UK, and 3,3',4',5-tetrachlorosalicylanilide (TCS) by Dr I. R. Booth, University of Aberdeen, UK.

Abbreviations: pH_o, extracellular pH; pH_i, intracellular pH; Na_o⁺, extracellular sodium concentration; Na_i⁺, intracellular sodium concentration; TCS, 3,3',4',5-tetrachlorosalicylanilide; CF, competence factor; $\Delta\mu_{H^+}$, electrochemical potential difference for protons; $\Delta\psi$, electric transmembrane potential; Δp H, pH_i – pH_o, i.e. the proton chemical gradient; F , Faraday's constant; Z , $2.3RT/F = 59$ mV at 25 °C.

Organism, growth media and competence development. The experiments were done with *S. pneumoniae* strain CP 1000 (Morrison *et al.*, 1984), which exhibits a wild-type phenotype for genetic transformation.

Bacteria were routinely induced to competence as described by Morrison *et al.* (1984). Briefly, stock cultures were grown at pH 7.5 in a complex medium containing (g l^{-1}): NaCl, 5; yeast extract, 1; tryptone, 5; enzymic casein hydrolysate, 10; glucose, 2; K_2HPO_4 , 3. When the culture reached an OD_{400} of about 0.4, glycerol (15%, v/v) was added and samples were kept frozen at -80°C . To obtain precompetent bacteria, samples were thawed and diluted 1 in 100 in standard growth medium, adjusted to pH 7 and enriched with CaCl_2 (1 mM) and bovine serum albumin (2 mg ml^{-1}), and were grown for 2 h at 37°C . Such cultures constituted the tester cells; when shifted to pH 7.8 they were activatable by 1–5% (v/v) of a crude preparation of CF or by 0.1–0.5% of a partly purified extract of CF obtained by $(\text{NH}_4)_2\text{SO}_4$ fractionation at pH 11. As the incubation time required to reach competence depends on parameters such as the culture density and the CF preparation, competence tests were routinely done in parallel with other measurements. Competence was monitored by assaying the appearance of DNA-degrading activity and by biological transformation tests. Selection of transformants for a given genetic marker after incubation with DNA was done as described by Morrison *et al.* (1984). Briefly, an appropriate dilution of the cell suspension that had been incubated with DNA bearing a genetic marker (*ami*) conferring methotrexate resistance (Mtx^R) was plated on blood-agar medium and incubated for 2 h at 37°C . A layer of agar containing the selective agent (10^{-5} M -methotrexate) was added and incubation continued. The number of colony forming units (c.f.u.) was determined after 18 h. The frequency of transformants was calculated as the ratio between the numbers of c.f.u. obtained in the presence and in the absence of the selective agent. Under our experimental conditions the number of spontaneous mutants never exceeded 1% of the number of transformants.

For NMR experiments, 500 ml of an early exponential phase culture, or 3 l of a culture induced to competence, was centrifuged at 4°C , washed with the buffer required for the NMR experiment, and concentrated in 1 ml of that buffer at 4°C . Competence tests and readings for NMR spectra (2–8 min accumulated free induction decay) were done immediately.

The medium used for NMR was derived from a classical mineral medium used for growth of *S. pneumoniae* (Trombe *et al.*, 1984). This medium allowed competence expression of induced bacteria but not competence induction: as far as could be determined, at least concerning competence, the physiological state of the culture at the time it was harvested for NMR measurements did not change during the experiment. The medium contained: Tris, 40 mM, NH_4Cl , 52 mM, KCl, 7.5 mM, MOPS, 20 mM. The pH and the NaCl concentration were adjusted as required. Osmolarity was maintained at 240 mosm by addition of KCl.

Internal pH (pH_i). (a) ^{31}P NMR combined with TCS treatment. The chemical shift of the inorganic phosphate peak can be taken as an indicator of the pH_i value; this allows one to ignore eventual differences between the internal volume of bacteria in different physiological states (Ogawa *et al.*, 1981; Slonczewski *et al.*, 1981; Booth, 1985). Indeed, for a given temperature, pH_i can be related to a Henderson–Hasselbach-type equation (Ogawa *et al.*, 1981; Lachmann & Schnackerz, 1984). When based on ^{31}P NMR, the method is limited to a pH range determined by the pK_2 of inorganic phosphate, the value of which depends on the ionic strength of the cytoplasm (Ogawa *et al.*, 1981). The chemical shift of the ^{31}P resonance is also influenced by the paramagnetic ionic content of the cells (Ezra *et al.*, 1983) and may vary between cultures. Therefore, pH_i was deduced from the external pH (pH_o) by means of a 'null-point' method after treatment of the bacteria with the protonophore TCS at different values of pH_o . TCS collapses the protonmotive force, i.e. $\Delta\psi$ and ΔpH . The remaining Donnan potential, which was about 11 mV in our experimental conditions, was constant throughout the experiment. Thus pH_i values estimated experimentally might differ from the real values by 0.1 pH unit. ^{31}P NMR measurements were done at 121.5 MHz on a Bruker AM 300WB instrument, using the standard 10 mm ^{31}P probe kept at 5°C by a stream of nitrogen. Experiments were done in flat-bottomed tubes, and the sample volume was adjusted to 2.3 ml. Bi-level high-power proton decoupling was used to remove ^1H – ^{31}P dipolar coupling (0.3 W between pulses and 4 W during data acquisition). Sample spinning was used to improve the resolution. Spectra were accumulated in 100–400 transients without ^2H lock using 7 μs (60°) pulses, an interpulse delay of 1.64 s, a sweep width of 10 KHz and 16K data points. An exponential multiplication corresponding to 80 Hz line-broadening was applied to the free induction decay to increase the signal-to-noise ratio and was zero-filled behind the Fourier transformation. Spectra were obtained after 2–8 min. NMR peak positions in spectra were given in parts per million (p.p.m.) from the peak position of 85% orthophosphoric acid as an external reference. The peaks were identified by their chemical shift. The assignments were confirmed by addition of the corresponding commercial compounds. For null-point determination, a ^{31}P NMR spectrum was obtained (Fig. 1) at a given pH_o and then TCS ($1\text{ }\mu\text{mol per } 5 \times 10^{10}$ bacteria) was added and a second spectrum was obtained. The displacement of the chemical shift (δ) of the phosphate resonance (^{31}P) induced by the protonophore was measured ($\Delta\delta$). An example is given in Fig. 2. The peak corresponding to inorganic phosphate was followed specifically because of its better resolution compared to the others. The null-point corresponds to conditions where $\Delta\delta$ was not significant.

(b) ΔpH determination by [^{14}C]benzoic acid accumulation. [^{14}C]Benzoic acid accumulation was determined as described previously (Trombe *et al.*, 1984), assuming an intracellular volume of $0.96\text{ }\mu\text{l per } 10^8$ bacteria (Trombe *et al.*, 1984). The pH_i was deduced from pH_o measurement and ΔpH calculation using steady-state accumulation of the probe.

Fig. 1

Fig. 2

Fig. 1. Typical ^{31}P NMR spectrum of *S. pneumoniae*, showing peaks representing (1) sugar phosphate, (2) inorganic phosphate, (3) teichoic acid and unidentified phosphate(s), (4) NAD/NADH, and (5) unidentified pyrophosphate compound(s). No difference was observed when spectra were performed between pH_o values of 7 and 8.5.

Fig. 2. TCS-induced displacement of the ^{31}P NMR spectrum of *S. pneumoniae*. First, the ^{31}P NMR spectrum of *S. pneumoniae* was obtained at pH_o = 7 (---), then TCS (1 μmol per 10^{10} bacteria) was added and a second spectrum was recorded (—). See text for details.

Determination of the internal Na^+ concentration (Na_i^+). (a) ^{31}P NMR combined with monensin treatment. Na_i^+ was deduced from Na_o^+ by means of a null-point method after treatment of the bacteria at different Na_o^+ values with monensin, an ionophore that exchanges monovalent cations for H^+ with a high specificity for Na^+ . Indeed, such a specificity was clear in our experiments where the pH_i shift induced by monensin addition responded to $\Delta\mu_{\text{Na}}$ rather than $\Delta\mu_{\text{K}}$. (see Tables 2 and 3). The operations were the same as for pH_i determination using ^{31}P NMR. An initial ^{31}P NMR spectrum was obtained at a given Na_o^+ value, then monensin (1 μmol per 5×10^{10} bacteria) was added and a second spectrum was obtained. The displacement of the chemical shift (δ) of the phosphate resonance (^{31}P) induced by monensin was measured ($\Delta\delta$). In the presence of monensin, Na^+ moves down its concentration gradient and the H^+ moves in the opposite direction. The null-point corresponds to conditions where $\Delta\delta$ is not significant and indicates that $\text{Na}_i^+ \approx \text{Na}_o^+$.

(b) ^{23}Na NMR. ^{23}Na NMR measurements were done at 66.15 MHz on a Bruker WH 250 instrument using the standard 10 mm ^{23}Na probe kept at 5 °C by a stream of nitrogen. Experiments were done in flat-bottomed tubes and the volume of the sample, bacteria suspended in a Na^+ -free medium, was adjusted to 1.5 ml. Spectra were accumulated in 500 transients using 7 μs (90°) pulses, an interpulse delay of 3 s, a sweep width of 50 kHz and 16K data points. The instrument was calibrated using NaCl solutions of known concentration. Relative quantities of free or bound Na^+ were evaluated either by measuring the areas of the peaks or by weighing after enlargement. Measurement accuracy was about $\pm 10\%$ for free Na^+ and 50% for the bound form.

Flame photometry. For measurement of ion contents by flame photometry (Schultz & Solomon, 1961), 10 ml samples of a culture were quickly filtered on HA Millipore filters pre-rinsed with 100 mM-(NH_4)₂SO₄. The bacteria were then washed with 2 ml of a solution of sucrose (500 mM) and the filters were eluted in 1 ml 1 M-HCl. Measurements were made using a Perkin-Elmer 290 B instrument. Control values were obtained by filtration of the culture medium; they never exceeded 20% of the sample value. Intracellular cation concentration was estimated assuming that 10^8 bacteria represent an intracellular space of 0.96 μl (Trombe *et al.*, 1984). The values presented are the means of four determinations on independent cultures. Each determination was the result of triplicate filtration assays on the same culture.

Glucose consumption. This was determined by measuring the amount of glucose present in the culture medium before and after bacterial growth using a commercial glucose oxidase/peroxidase system (Merck).

ATP and L-lactate determinations. These were done after perchloric acid extraction as described previously (Trombe *et al.*, 1984). Briefly, 1 ml of a culture in the required physiological state was precipitated with 90 μl 70% (v/v) perchloric acid at 4 °C. After centrifugation, 900 μl of supernatant was withdrawn and neutralized with 215 μl of a solution containing 1.2 M-Tris, 0.175 M-KCl, 4 M-KOH. The sample was then centrifuged and the supernatant was kept frozen before ATP and L-lactate determinations. ATP was assayed by bioluminescence

measurements using a luciferin-luciferase system (Lumit-PM). Calibration was by adding known amounts of ATP to the samples. For each sample ten determinations were made; the six to nine values closest to the mean were used. Standard deviations varied from 2 to 10% of the mean. The quantity of L-lactate produced during bacterial growth was determined using commercial L-lactate dehydrogenase from rabbit muscle.

$\Delta\psi$ measurement. This was done by measuring [^3H]tetraphenylphosphonium accumulation and using the Nernst equation as described elsewhere (Trombe *et al.*, 1984).

RESULTS

Protonmotive force at competence

If not otherwise stated, measurements were made during competence development and more precisely at the peak time, when the frequency of competent bacteria in the population reached 0.9 as shown by transformation experiments using a cloned genetic marker (*ami*) (see Fig. 3).

In order to determine the reason for increased isoleucine transport at competence (Trombe, 1983) both components of the protonmotive force were measured. The value obtained for $\Delta\psi$ was $138 \text{ mV} \pm 5 \text{ mV}$, which is similar to previous determinations in these bacteria (Trombe *et al.*, 1984). On the other hand, measurements using [^{14}C]benzoic acid gave a ΔpH value of 0.5 at $\text{pH}_o = 7.8$, indicating a net alkalization of the cytoplasm at competence since the control exhibited no ΔpH in the same conditions, as already shown in *S. pneumoniae* (Trombe *et al.*, 1984). This result was confirmed by the method combining ^{31}P NMR and TCS treatment (see Methods). TCS induced an acidification of the cytoplasm in the range of $7 < \text{pH}_o < 8.2$, with a null point between 8.2 and 8.4 in competent bacteria, while in the control the null point occurred at $\text{pH}_o = 7.9$ (Table 1). It is important to note that, in competent *S. pneumoniae*, a ^{31}P NMR resonance shift after TCS addition was still observed at $\text{pH}_o = 8.2$. According to several reports, the $\text{p}K_2$ of inorganic phosphate is 6.8 at physiological ionic strength (Nicolay *et al.*, 1981; Ogawa *et al.*, 1981). Under these conditions, ^{31}P NMR resonance is invariant above pH 8. The shift, still observed at $\text{pH}_o = 8.2$, suggests that, in *S. pneumoniae*, the $\text{p}K_2$ of inorganic phosphate was more alkaline than in other neutrophilic bacteria studied (Nicolay *et al.*, 1981; Slonczewski *et al.*, 1981). It is possible that a more alkaline $\text{p}K_2$ reflects a lower ionic strength of the cytoplasm of *S. pneumoniae* compared to other species. Unfortunately, we were unable to construct a titration curve in order to determine $\text{p}K_2$, because the intracellular volume contribution to the total volume of a lysate could not exceed 10%, given the known intracellular space of the bacteria (0.96 μl for 10^8 cells; Trombe *et al.*, 1984).

Thus competent bacteria have a cytoplasm which is more alkaline than that of the controls, and as a consequence a higher $\Delta\mu_{\text{H}^+}/F$ resulting from an extra ΔpH of 30 mV.

Na^+ and K^+ content at competence

The Na^+ and K^+ contents of the culture during the development of competence were measured by flame photometry. There was no significant variation in the K^+ content of the culture, suggesting no specific regulation of K^+ transport during competence as previously shown by Tomasz (1969) (Table 2). In contrast, the values for Na^+ content fluctuated with a maximum around 340 mM before the start of competence (15 min after CF addition), 120 mM at the peak time and 86 mM at the end of the competence wave (Table 2). This last value was similar to that in the control (not shown).

These results were corroborated by determinations using a method where ^{31}P NMR and monensin treatment were combined. Monensin, an ionophore that exchanges H^+ and cations with a high specificity for Na^+ , was expected to change pH_i while it reduced $\Delta\mu_{\text{Na}^+}$. Thus, shifts in ^{31}P NMR displacement after monensin addition should reflect monensin-induced pH_i changes consequent upon the existence of a transmembrane Na^+ gradient. In the absence of a Na^+ gradient no change of pH_i should occur. Na^+ may therefore be estimated from the Na_o^+ value under conditions where no ^{31}P NMR resonance displacement was observed after monensin addition. The results of a typical experiment using non-competent bacteria (Table 3) showed that for Na_o^+ values ranging between 1 and 60 mM the ^{31}P NMR peak shifted toward positive values after monensin addition, indicating an acidification of the cytoplasm. At

Table 1. TCS-induced displacement of ³¹P NMR resonance at different pH_o values in competent and non-competent bacteria

pH _o *	Δδ (p.p.m.)†	
	Non-competent bacteria	Competent bacteria
7.0	+0.45 ± 0.05*	+0.49
7.5	+0.16	ND
7.8	+0.06	+0.21 ± 0.05*
8.0	-0.01	+0.17 ± 0.09*
8.2	ND	+0.07 ± 0.05*
8.4	0.00	-0.03

ND, Not determined.

* pH of the bacterial suspension.

† δ(P_i) shift induced by TCS addition (1 μmol per 5 × 10¹⁰ bacteria). Where ± terms are shown, these are standard deviations of three independent determinations.Table 2. Bacterial Na⁺ and K⁺ content during a competence cycle

Time* (min)	Intracellular concn† (mM) of:		DNA degradation‡ (d.p.m. ml ⁻¹)
	Na ⁺	K ⁺	
5	45 ± 10	272 ± 2	0
10	210 ± 20	ND	0
15	339 ± 39	283 ± 2	0
20	153 ± 12	229 ± 14	910
25	121 ± 7	250 ± 8	3000
38	121 ± 2	274 ± 10	26940
50	88 ± 6	206 ± 19	31600
60	78 ± 7	223 ± 24	31860

ND, Not determined.

* Incubation after addition of CF (5%, v/v) to the culture. In these conditions the competence peak occurs between 25 and 38 min.

† The results are means ± standard deviation of four independent determinations.

‡ [³H]DNA (4.2 × 10⁴ d.p.m. ml⁻¹) was added to a sample, and competence development was monitored by assaying DNA degrading activity. The results are means of three determinations; values varied by ± 10% about the mean.Table 3. Monensin-induced displacement of ³¹P NMR resonance in competent and non-competent bacteria at different Na_o⁺ values

Na _o ** (mM)	Δδ (p.p.m.)†	
	Non-competent bacteria	Competent bacteria
<1	+0.75	+0.468
60	+0.14	+0.428
85	-0.04 ± 0.05	+0.34
120	-0.26	-0.02 ± 0.08
160	ND	-0.29

ND, Not determined.

* Na⁺ concentration of the bacterial suspension.† δ(P_i) shift induced by Monensin addition (1 μmol per 5 × 10¹⁰ bacteria). Where ± terms are shown, these are standard deviations of three independent determinations.

Fig. 3. ATP content of the culture during competence expression. Tester cells were incubated at 37 °C in the presence or in absence of CF (5%, v/v). At intervals, 1 ml samples were extracted for ATP determination (vertical bars: —, plus CF; ---, minus CF), and 0.1 ml samples were incubated for 5 min at 30 °C in the presence of pR172 DNA (0.1 µg ml⁻¹) bearing the *ami* marker. The frequency of Mtx^R transformants (●) was determined by plating (see Methods). ○, C.f.u. ml⁻¹ in the absence of CF; ●, c.f.u. ml⁻¹ in the culture induced by CF.

120 mM-Na_o⁺, the ³¹P NMR resonance peak shifted toward negative values, suggesting alkalinization of the cytoplasm. The null point (Na⁺ concentration equal in the intracellular and extracellular compartments) occurred at 85 mM-Na_o⁺ (Table 3). In contrast, when competent bacteria were used, the null point occurred at 120 mM (Table 3). These results were in good agreement with data obtained by flame photometry (Table 2); both approaches showed that competent bacteria had a greater Na⁺ pool than non-competent bacteria. Indeed, ²²Na transport measurements indicated a net uptake of Na⁺ in competent bacteria compared to controls (not shown).

Na⁺ can be free in the cytoplasm or it may be linked to macromolecular species as revealed by ²³Na NMR (Lazlo, 1978). ²³Na NMR of control and competent bacteria showed a similar partitioning of Na⁺, with a ratio of free Na⁺ to bound Na⁺ of about 0.7, suggesting no specific change in the distribution of the cation within the cytoplasm at competence.

In order to check the physiological significance of the observed modulation of Na⁺ transport during competence induction we used monensin at Na_o⁺ = 120 mM. A concentration as low as 2 µM-monensin totally inhibited competence induction without reducing cell viability. This suggests that a controlled Na⁺ circulation is determining induction.

Competence cycle and the ATP pool

ATP measurements in bacterial extracts prepared at different stages of competence expression showed that in competent bacteria the ATP pool reached 9.5 ± 0.5 mM while it was 6.3 ± 0.5 mM in the control (Fig. 3). This enhanced ATP content probably resulted from glycolytic stimulation; glucose consumption in competent cultures was 50% greater than that in control cultures (330 vs 220 µmol per OD₄₀₀ unit).

DISCUSSION

Several independent methods showed an increased intracellular pH associated with an enhanced Na⁺ content of the bacteria at competence.

The results obtained by Δ pH determination using [¹⁴C]benzoic acid as a probe agreed well with those obtained by a combination of ³¹P NMR resonance and protonophore treatment at different pH_o values. They indicated a net alkalization of the cytoplasm in competent bacteria: the pH_i of such cells reached 8.3, and created a Δ pH of 0.5 at pH_o 7.8, while in the control, pH_i was 7.8 and no Δ pH was measured, a result similar to previous findings (Trombe *et al.*, 1984). No change in $\Delta\psi$ value was observed at competence, therefore the increased Δ pH might account for the increased rate of Δ p-dependent isoleucine uptake in competent bacteria (Trombe, 1983).

Direct Na⁺ determinations by flame photometry corroborated results derived from a combination of ³¹P NMR and monensin utilization at different Na_o⁺ values. The Na_i⁺ value was about 120 mM in competent bacteria, compared to 85 mM in the control. This increase in Na⁺ was not followed by a change in the distribution of the cation within the bacteria as shown by ²³Na NMR, a technique which allowed estimation of the ratio of free Na⁺ to bound Na⁺; this ratio was around 0.7 in both competent and control bacteria. Uptake experiments using ²²Na suggested that this increase resulted from net uptake of Na⁺ (not shown). This increase in pH_i and Na_i⁺ was not accompanied by variation of the K⁺ content of the bacteria during the competence cycle (Table 2). However, it was accompanied by a stimulation of glycolysis resulting in an enhanced ATP content during induction (Fig. 3).

It is possible that cytoplasmic alkalization resulting from Na⁺/H⁺ exchange stimulated glycolysis (Moore *et al.*, 1979). A Na⁺/H⁺ antiporter has been described in several bacteria, including *E. coli* (West & Mitchell, 1974; Bassilana *et al.*, 1984). Activation of such a function at competence should increase pH_i and Na_i⁺ and stimulate glycolysis, as already shown in some eukaryotic cells stimulated by insulin (Moore *et al.*, 1979) or growth factors (Shuldiner & Rozengurt, 1982; Moolenaar *et al.*, 1983; Paris & Pouyssegur, 1984; Metcalfe *et al.*, 1985). However, such functioning of the Na⁺/H⁺ antiporter in eukaryotes is dependent upon the generation of a Na⁺ gradient by the Na⁺/K⁺ ATPase (Rozengurt, 1980). A similar enzymic function has been proposed in *S. faecalis* (Kakinuma & Harold, 1985) but has yet to be established in *S. pneumoniae*. Homeostatic regulation in prokaryotes is more complex (Plack & Rosen, 1980; Kroll & Booth, 1981; Kobayashi *et al.*, 1982; Booth, 1985), since pumps exchanging Na⁺/H⁺ (Heefner & Harold, 1982) and Na⁺/K⁺ (Kakinuma & Harold, 1985), as well as the Na⁺/H⁺ antiporter (West & Mitchell, 1974; Bassilana *et al.*, 1984; Goldberg *et al.*, 1987), contribute to the production of a Na_i⁺/Na_o⁺ gradient < 1 (Schultz & Solomon, 1961; Castle *et al.*, 1986) while pH_i seems to be regulated only by the F₁F_o ATPase in streptococci (Kobayashi *et al.*, 1982).

An increase in ATP produced by glycolysis was described in the SOS response in *E. coli* (Barbé *et al.*, 1983) and an SOS-like system is induced at competence in *Bacillus subtilis* (Love & Yasbin, 1986). On the other hand, a similar response was obtained in *S. pneumoniae* in the late exponential growth phase (not shown). Thus the stimulation of glycolysis may constitute a metabolic response to some stress or/and nutritional imbalance. At competence the regulation of Na⁺ fluxes appeared to be directly involved in the induction process since 2 μ M-monensin, which tends to collapse $\Delta\mu_{Na^+}$, totally blocked induction without inhibiting growth. Moreover, cytoplasmic alkalization at competence probably corresponds to optimal conditions for DNA uptake (Clavé *et al.*, 1987). Experiments are now in progress to characterize the functions, probably under the control of competence activator, that are involved in such regulation.

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