ACTA BIOCHIMICA POLONICA
Vol. 39 1992 No. 1

ZOFIA TYNECKA and ANNA MALM

EFFECT OF Cd^{2+} ON PHOSPHATE EXCHANGE IN *STAPHYLOCOCCUS AUREUS*

Department of Pharmaceutical Microbiology, Medical Academy, Lubartowska 85, 20 - 123 Lublin, Poland

Bacteria take up inorganic phosphate (P_i) *via* several transport systems [1]. According to Mitchell & Moyle [2], in growing cells of *Staphylococcus aureus* the internal P_i pool of about 0.1 M [3] is utilized for P_i incorporation into various compounds. The P_i which has been incorporated is continously replaced by transport of external P_i. Thus, only an inward movement of P_i in growing cells of this organism takes place [2]. In contrast, in resting cells of *S. aureus*, a bidirectional exchange of P_i across the osmotic barrier occurs due to limitation of P_i incorporation in the absence of growth [2, 3, 4]. As found by Mitchell & Moyle [2], P_i was taken up across the osmotic barrier at the same rate in growing and resting cells of *S. aureus*.

We have shown by inhibitor and ionophore studies that in growing cells of the cadmium-sensitive *S. aureus* 17810S oxidizing exogenous amino acids, the initial uptake of $^{32}P_i$ occurred *via* a P_i carrier down the pH gradient (ΔpH) generated by the respiratory chain [5]. In these cells 10 µM Cd^{2+} strongly inhibited both respiration and $^{32}P_i$ uptake, which suggested that Cd^{2+} could block P_i influx at the level of ΔpH formation [5]. The aim of the present paper was to study the effect of Cd^{2+} on $^{32}P_i$ uptake in resting cells of *S. aureus* 17810S oxidizing endogenous amino acids in the absence of an exogenous energy source [6].

Resting cells of *S. aureus* 17810S suspended in 1 or 100 mM potassium/sodium phosphate buffer, pH 7, were obtained as described previously

[7]. $^{32}P_i$ (20 μCi/μmol) was added to cell suspensions, and samples were withdrawn at indicated time intervals, then filtered through membrane filters of 0.45 μm (Sartorius) and immediately washed with 5 ml of the same buffer. The radioactivity on filters was measured in a scintillation counter Intertechnique SL, France. Oxygen uptake was assayed manometrically according to [8].

Figure 1A presents $^{32}P_i$ uptake, at 1 mM external concentration, in resting cells of strain 17810S. $^{32}P_i$ uptake did not achieve a steady-state equilibrium even in 50 min. At higher external $^{32}P_i$ concentration (100 mM), the steady-state equilibrium was attained as soon as after 20 min followed by $^{32}P_i$ escape (Fig. 1B). Addition of 100 mM unlabelled P_i at 10 min to cells which had taken up $^{32}P_i$ from 1 mM solution resulted in efflux

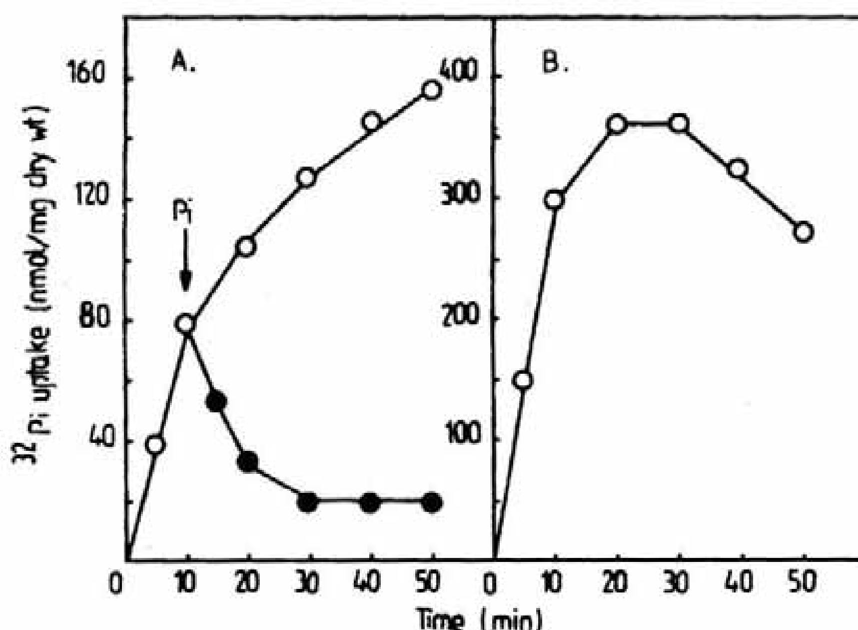

Fig. 1. Time course of $^{32}P_i$ uptake in resting cells of *S. aureus* 17810S. A, two batches of cell suspension in 1 mM potassium/sodium phosphate buffer, pH 7, were incubated on a shaker with $^{32}P_i$ at 37°C (○). To one batch of the suspension, 100 mM unlabelled P_i was added at the indicated time (arrow). B, cell suspension in 100 mM potassium/sodium phosphate buffer, pH 7, was incubated on a shaker with $^{32}P_i$ at 37°C (○). The results are representative of 3 experiments

of the labelled P_i (Fig. 1A). This indicates that in resting cells of strain 17810S a bidirectional movement of phosphate across the membrane took place.

Figure 2 shows the effects of various inhibitors and ionophores on $^{32}P_i$ uptake in resting cells of strain 17810S. At 10 µM concentration *p*-

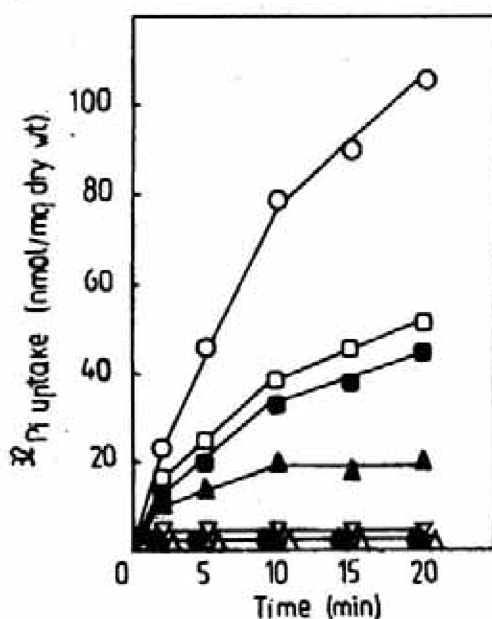

Fig. 2. Effects of inhibitors and ionophores on $^{32}P_i$ uptake in resting cells of *S. aureus* 17810S. Cell suspensions in 1 mM potassium/sodium phosphate buffer, pH 7, were pretreated, before addition of $^{32}P_i$ for 10 min at 37°C with: △, 10 µM pCMB; ▲, 10 mM sodium arsenate; □, 4 µM nigericin; ■, 50 µM CCCP; ▽, 4 µM nigericin + 10 µM pCMB; ○, control cells at 37°C; ●, cells at 4°C. Results are mean of 3 determinations

chloromercuribenzoate (pCMB), an inhibitor of the P_i porter in bacteria [1] and in mammalian mitochondria [9], abolished $^{32}P_i$ uptake in strain 17810S, as did low temperature (Fig. 2). Also 10 mM arsenate, a competitive inhibitor of some P_i transport systems [1], strongly inhibited $^{32}P_i$ uptake in this organism (Fig. 2). Pretreatment of the cells with 4 µM nigericin, which selectively collapses ΔpH [10], or with 50 µM carbonylcyanide *m*-chlorophenylhydrazone (CCCP), which leads to a discharge of the total electrochemical gradient of protons $-\Delta\tilde{\mu}_{H^+}$ [10], resulted in reduction of the initial rate of $^{32}P_i$ uptake in strain 17810S (Fig. 2). However, this uptake showed a tendency to increase with time (Fig. 2). The reduced $^{32}P_i$

uptake in nigericin-pretreated cells of this organism was abolished by pCMB (Fig. 2).

As shown in Fig. 3, 100 µM 2-heptyl-4-hydroxy-quinoline *N*-oxide (HQNO), an inhibitor of staphylococcal electron transfer chain [11] had no

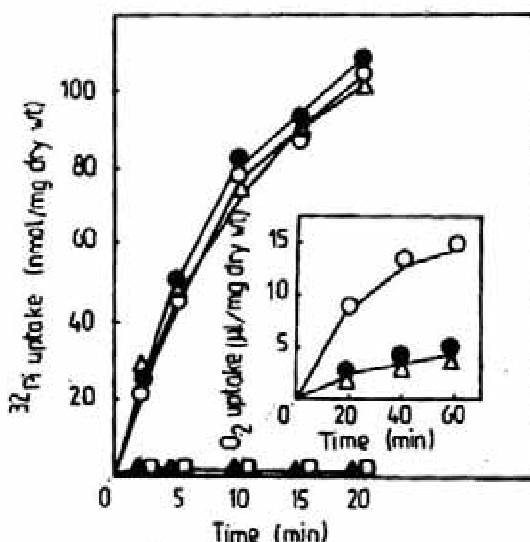

Fig. 3. Effects of HQNO and Cd^{2+} on $^{32}P_i$ uptake in resting cells of *S. aureus* 17810S. Cell suspensions in 1 mM potassium/sodium phosphate buffer, pH 7, were pretreated, before addition of $^{32}P_i$ for 10 min at 37°C with: ●, 100 µM HQNO; Δ, 10 µM Cd^{2+}; ▲, 100 µM HQNO + 10 µM pCMB; □, 10 µM Cd^{2+} + 10 µM pCMB; ○, control cells. Inset: effects of HQNO and Cd^{2+} on endogenous respiration of *S. aureus* 17810S. o, control cells; cells pretreated for 10 min at 37°C with ●, 100 µM HQNO; Δ, 10 µM Cd^{2+}. Results represent the mean of 3 experiments

effect on $^{32}P_i$ uptake in resting cells of strain 17810S, although it strongly inhibited oxidation of endogenous amino acids (Fig. 3, inset). $^{32}P_i$ uptake in the presence of HQNO was abolished by pCMB (Fig. 3). Cd^{2+} (10 µM) which enters *S. aureus* 17810S cells *via* the Mn^{2+} transport system down a membrane potential gradient $-\Delta\phi$ [12, 13] did not affect $^{32}P_i$ uptake in resting cells of this organism either (Fig. 3), although it strongly inhibited endogenous respiration (Fig. 3, inset). $^{32}P_i$ uptake in the Cd^{2+}-pretreated cells of strain 17810S was blocked by pCMB (Fig. 3).

The data presented in this paper indicate that in resting cells of *S. aureus* 17810S $^{32}P_i$ uptake represents an exchange of external $^{32}P_i$ with the internal P_i pool *via* the P_i porter which is sensitive to pCMB, arsenate and low temperature. Partial reduction of P_i exchange by nigericin or CCCP suggests

that ΔpH, preformed during cell growth, was required for formation and maintenance of the internal P_i pool needed for P_i exchange. It is probable that nigericin or CCCP reduced the internal P_i pool by efflux of P_i and this could decrease P_i exchange. In the absence of ΔpH dissipated by nigericin, reduced P_i exchange occurred *via* the P_i porter, sensitive to pCMB.

In the absence of endogenous respiration blocked either by Cd^{2+} or by HQNO, which do not discharge ΔpH but only prevent its formation, a normal P_i exchange, sensitive to pCMB, took place. This suggests that P_i exchange does not require continuous formation of ΔpH by the respiratory chain. To summarize, P_i exchange in resting cells of strain 17810S requires an intact P_i porter and maintenance of the internal P_i pool. In contrast, in growing cells of this organism initial $^{32}P_i$ uptake representing an unidirectional P_i influx, required both the intact P_i porter and also formation of ΔpH by the respiratory chain, since this $^{32}P_i$ uptake was blocked by pCMB, arsenate, low temperature, nigericin, CCCP, HQNO, anoxia and Cd^{2+} [5]. Our data indicate also that Cd^{2+}, unlike Hg^{2+}, has no deleterious effects on the P_i porter in *S. aureus*. This confirms our previous suggestion [5] that in growing cells of strain 17810S Cd^{2+} inhibited $^{32}P_i$ uptake at the level of energy generation but not due to blocking of the P_i porter.

REFERENCES

1. Rosenberg, H. (1987) in, *Ion transport in prokaryotes* (Rosen, B. P. & Silver, S., eds.) pp. 205 - 248. Academic Press, Inc. New York.
2. Mitchell, P. & Moyle, J. M. (1953) *J. Gen. Microbiol.*, **9**, 257 - 272.
3. Mitchell, P. (1953) *J. Gen. Microbiol.*, **9**, 273 - 287.
4. Mitchell, P. (1954) *J. Gen. Microbiol.*, **11**, 73 - 82.
5. Tynecka, Z. & Szcześniak, Z. (1991) *Microbios.*, **68**, 53 - 63.
6. Tynecka, Z. (1968) *Exp. Med. Microbiol.*, **20**, 228 - 232.
7. Tynecka, Z., Malm, A., Skwarek, T. & Szcześniak, Z. (1989) *Acta Microbiol. Polon.*, **38**, 58 - 75.
8. Tynecka, Z., Zając, J. & Goś, Z. (1975) *Acta Microbiol. Polon.*, **7**, 11 - 20.
9. Fonyo, A. (1979) *Pharmac. Ther.*, **7**, 627 - 645.
10. Mitchell, P. (1966) *Biol. Rev.*, **41**, 445 - 502.
11. Taber, H. W. & Morrison, M. (1964) *Arch. Biochem. Biophys.* **105**, 367 - 379.
12. Tynecka, Z., Goś, Z. & Zając, J. (1981) *J. Bacteriol.*, **147**, 305 - 312.
13. Tynecka, Z., Malm, A. & Zając, J. (1990) *Acta Biochim. Polon.*, **37**, 121 - 124.