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

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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 continuously 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 $^{32}$P$_i$ (20 μCi/μmol) was added to cell suspensions, and samples were withdrawn at indicated time intervals, then filtered through membrane filters of 0.45 μm (Sartorius) and immediately washed with 5 ml of the same buffer.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 ^32P_i uptake in resting cells of strain 17810S. At 10 µM concentration p-

![Graph](image)

**Fig. 2.** Effects of inhibitors and ionophores on ^32P_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 ^32P_i for 10 min at 37°C with: Δ, 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.

chloromercuronbenzoate (pCMB), an inhibitor of the P_i porter in bacteria [1] and in mammalian mitochondria [9], abolished ^32P_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 ^32P_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 −Δ\(\mu\)H^+ [10], resulted in reduction of the initial rate of ^32P_i uptake in strain 17810S (Fig. 2). However, this uptake showed a tendency to increase with time (Fig. 2). The reduced ^32P_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 effect on $^{32}\text{P}_i$ uptake in resting cells of strain 17810S, although it strongly inhibited oxidation of endogenous amino acids (Fig. 3, inset). $^{32}\text{P}_i$ 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 $^{32}\text{P}_i$ uptake in resting cells of this organism either (Fig. 3), although it strongly inhibited endogenous respiration (Fig. 3, inset). $^{32}\text{P}_i$ 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 $^{32}\text{P}_i$ uptake represents an exchange of external $^{32}\text{P}_i$ with the internal $\text{P}_i$ pool via the $\text{P}_i$ porter which is sensitive to pCMB, arsenate and low temperature. Partial reduction of $\text{P}_i$ exchange by nigericin or CCCP suggests...
that ΔpH, preformed during cell growth, was required for formation and maintenance of the internal P\textsubscript{i} pool needed for P\textsubscript{i} exchange. It is probable that nigericin or CCCP reduced the internal P\textsubscript{i} pool by efflux of P\textsubscript{i} and this could decrease P\textsubscript{i} exchange. In the absence of ΔpH dissipated by nigericin, reduced P\textsubscript{i} exchange occurred via the P\textsubscript{i} porter, sensitive to pCMB.

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

REFERENCES