# PHOSPHATE TRANSPORT IN MEMBRANE VESICLES OF *PARACOCCUS DENITRIFICANS*

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## 1. Introduction

The mitochondrial $P_i$ carrier mediates the electroneutral, reversible exchange of $P_i$ for $OH^-$ across the inner mitochondrial membrane [1,2]. Thus $P_i$ uptake into the mitochondrion occurs primarily in response to the pH gradient (alkaline inside) imposed across the mitochondrial membrane [3]; this is effectively the proton symport of Mitchell [4].

In bacteria the mechanism of $P_i$ uptake is less well understood. In *Staphylococcus aureus* and *Escherichia coli* [4] and in *Streptococcus faecalis* [5] it has been proposed that $P_i$ uptake occurs by an electroneutral exchange of $P_i$ for $OH^-$ across the bacterial plasma membrane, but in none of the bacteria so far investigated has $P_i$ uptake in response to a pH gradient been demonstrated.

The plasma membrane of *Paracoccus denitrificans*, previously *Micrococcus denitrificans* [6], is already known to possess many features of the mitochondrial inner membrane [7], and this prompted us to determine whether *P. denitrificans* also possesses the same type of $P_i$ carrier as the mitochondrion.

In the present paper we show that $P_i$ uptake into membrane vesicles prepared from the plasma membrane of *P. denitrificans* can be driven by a pH gradient (alkaline inside) applied across the vesicle membrane. We also show that the $P_i$ carrier present in these vesicles (again like the mitochondrial $P_i$ carrier) is reversible and sensitive to SH-group reagents.

*Abbreviations:* FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; $P_i$, orthophosphate; PMS, phenazine methosulphate.

$P_i$ uptake into bacterial membrane vesicles has not previously been reported [8].

In a previous paper [9] we have demonstrated sulphate uptake into membrane vesicles of *P. denitrificans* under essentially the same conditions as those employed in the present paper to demonstrate $P_i$ uptake.

## 2. Materials and methods

*P. denitrificans* (*Micrococcus denitrificans* NCIB 8944) was grown in liquid culture, inside out vesicles were prepared from cells grown with succinate and nitrate, and right-side out vesicles were prepared from cells grown with $H_2$ and $O_2$, all as described previously [9]. Evidence supporting the description of the vesicles as inside out or right-side out is provided elsewhere [9]. $P_i$ uptake was measured by the procedure described previously for sulphate uptake [9], except that 1 $\mu$mol [$^{32}$P]$P_i$ (0.25 $\mu$Ci/$\mu$mol) was substituted for $Na_2$ [$^{35}$S]$SO_4$. Sulphate uptake was measured as described previously [9].

## 3. Results

Right-side out vesicles accumulated $P_i$ in the presence of either ascorbate and PMS (fig.1A) or succinate (fig.1B) as respiratory substrates. Inside out membrane vesicles however did not accumulate $P_i$ under these conditions (fig.1). The uptake of $P_i$ into right-side out vesicles was dependent upon the presence of a respiratory substrate and was abolished by the inclusion in the reaction medium of the un-

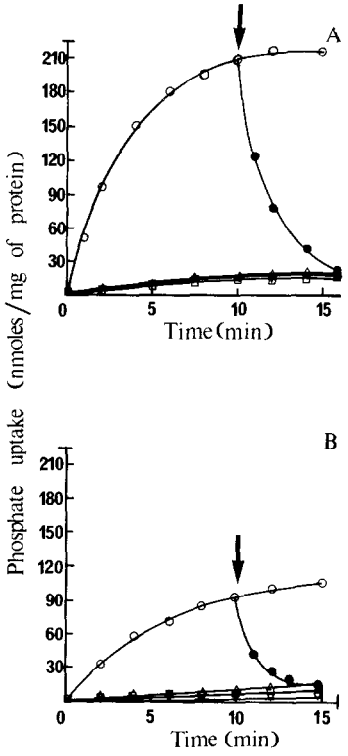


Fig.1. Respiration-driven $P_i$ uptake in membrane vesicles of *P. denitrificans*. $P_i$ uptake into right-side out (○, ●, □) and inside out (△, ▽) vesicles was measured in a 2 ml incubation mixture which contained: 1 μmol [$^{32}$P]$P_i$ (0.25 μCi/μmol), 0.2 ml of the vesicle suspension (1.5 mg protein), 100 μmol Tris–Cl (pH 7.3) and either 20 mM sodium ascorbate plus 0.1 mM PMS (A), or 10 mM sodium succinate (B). FCCP (5 μM) was either omitted (○, △) included in the incubation mixture (□, ▽), or added at time 10 min (●).

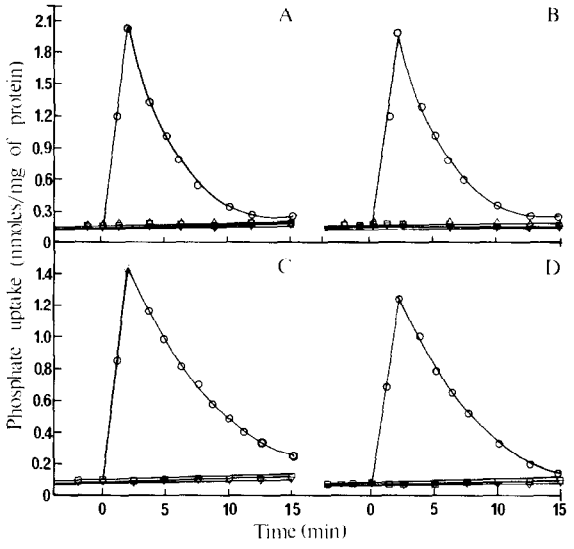


Fig.2. Transient uptake of $P_i$ into membrane vesicles of *P. denitrificans* driven by the addition of salts. $P_i$ uptake into right-side out (A,C) and inside out (B,D) vesicles was measured in a 2ml incubation mixture which contained: 1 μmol [$^{32}$P]$P_i$ (0.25 μCi/μmol), 0.2 ml of the vesicle suspension (1.5 mg protein) and 100 μmol Tris-Cl (pH 7.3). At time 0 min either 150 mM KCl (A,B) or 150 mM $NH_4Cl$ (C,D) were added. In A and B 1 μg nigericin was included in the incubation mixture. Further additions and subtractions to the incubation mixture were made as follows: none (○), plus 5 μM FCCP (□), minus nigericin (△), plus 1 μg valinomucin, minus nigericin (▽).

coupling agent FCCP (fig.1). When this uncoupler was added to right-side out vesicles which had already accumulated $P_i$ there was a rapid efflux of the accumulated $P_i$ (fig.1).

In the absence of a respiratory substrate an addition of 150 mM KCl to a suspension of the membrane vesicles which contained nigericin resulted in a transient uptake of $P_i$ (figs. 2A,B). This transient uptake occurred to a similar extent when either right-side out (fig.2A) or inside out (fig.2B) vesicles were used. No uptake was observed when nigericin was omitted, or when nigericin was substituted by valinomycin (figs.2A,B). Addition of 150 mM $NH_4Cl$ also resulted in a transient uptake of $P_i$ and, again, this uptake occurred to a similar extent when either right-side out (fig.2C) or inside out (fig.2D) vesicles were used.

Inclusion of the SH-group reagents *N*-ethylmaleimide and methylmercuric chloride prevented both the KCl- and $NH_4Cl$-induced $P_i$ uptake into either right-side out or inside out vesicles. Addition of these inhibitors to vesicles which had already taken up $P_i$ in response to an addition of KCl (in the presence of nigericin) decreased the rate at which $P_i$ was subsequently lost from the vesicles (fig.3). This decrease was observed with both right-side out (fig.3A) and inside out (fig.3B) vesicles.

Fig.4 shows that the substitution of cysteine for sulphate in the growth medium caused a total loss of the ability of the resulting vesicles to take up sulphate, but did not affect their ability to take up $P_i$. Cells

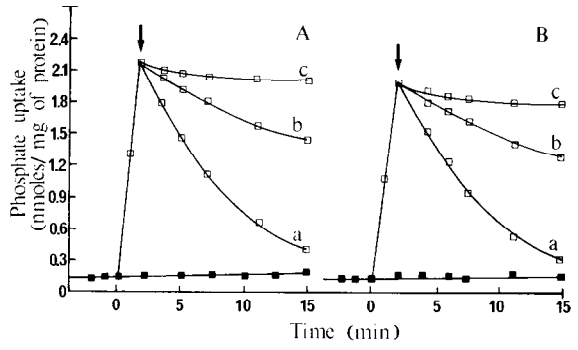


Fig.3. Effect of SH-group reagents on the efflux of $P_i$ transiently accumulated by membrane vesicles of *P. denitrificans*. Uptake of $P_i$ into right-side out (A) and inside out (B) vesicles was driven by the addition of 150 mM KCl in the presence of 1 μg nigericin as in figures 2A,B. FCCP (5 μm) was included in (■) or omitted from the incubation mixture as indicated. At time 2 min (indicated by arrow) further additions were made as follows: none (a), 10 mM *N*-ethylmaleimide (b), and 10 mM methylmercuric chloride (c).

grown on cysteine as the sole source of sulphur lack the sulphate carrier. The ability of vesicles prepared from cells grown on cysteine to take up $P_i$ but not sulphate shows clearly that $P_i$ and sulphate have different carriers.

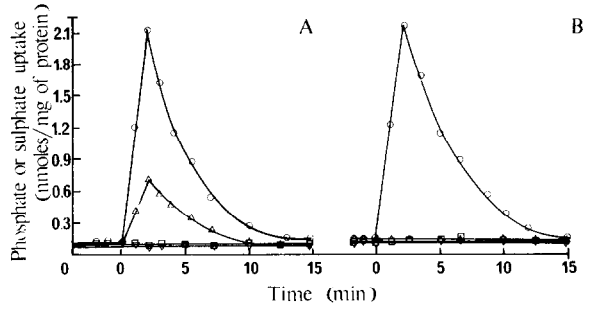


Fig.4. Effect of sulphur source during growth of cells on $P_i$ and sulphate uptake into membrane vesicles of *P. denitrificans*. Inside out vesicles were prepared from cells grown with succinate as substrate, nitrate as terminal electron acceptor, and either sulphate (A) or 2 mM cysteine (B) as the sulphur source. The transient uptake of $P_i$ (○) or sulphate (△) was driven by an addition of 150 mM-KCl in the presence of 1 μg nigericin as in figure 2B, in the presence (□, ▽) or absence (○, △) or 5 μM FCCP.

## 4. Discussion

It may be concluded from the present demonstration of $P_i$ uptake into membrane vesicles of *P. denitrificans* that $P_i$ transport in this bacterium, unlike $P_i$ transport in this bacterium, unlike $P_i$ transport in *E. coli* [10], does not require a periplasmic binding protein, since the preparation of membrane vesicles always appears to result in the loss of such proteins [11].

As suggested previously [9] the addition either of KCl (in the presence of nigericin) or of $NH_4Cl$ generates a pH gradient (alkaline inside) across the vesicle membrane by removal of protons from the vesicle interior, either by electroneutral exchange for $K^+$, or by combination with $NH_3$ to give $NH_4^+$. This pH gradient drives $P_i$ uptake as proposed by the chemiosmotic theory [4]. The detailed application of this theory for the uptake of other substrates has been discussed by Harold [12,13] and Hamilton [14]. The ability of SH-group reagents to trap the $P_i$ already taken up by the vesicles indicates that uptake is mediated by a carrier sensitive to SH-group reagents. The reversibility of this carrier is indicated principally by its ability to mediate $P_i$ uptake either into right side out or into inside out vesicles.

We therefore conclude that $P_i$ uptake into vesicles of *P. denitrificans* does not require a periplasmic binding protein, is driven by a pH gradient, and involves a carrier which is sensitive to SH-group reagents and which is reversible. In all these respects $P_i$ transport in vesicles from *P. denitrificans* resembles mitochondrial $P_i$ transport.

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