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THE MOVEMENT OF CATIONS CONNECTED WITH METABOLISM IN *AZOTOBACTER VINELANDII*

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SUMMARY

1. The movement of K^+ and the production of protons during metabolism have been studied in *Azotobacter vinelandii.*

2. During oxidation of Krebs-cycle intermediates protons are liberated in the medium with the same kinetics as that of the oxygen uptake. The protons are due to the CO_2 formed.

3. K^+ is taken up during metabolism of all Krebs-cycle intermediates. After anaerobiosis leakage is negligible unless valinomycin or nigericin are present.

4. There is a linear relationship between the rate and the extent of the K^+ uptake.

5. Cations stimulate the oxidation of Krebs-cycle intermediates by acting at the outside of the cell. They change the maximum velocity but not the K_m of substrate for oxidation. K^+ is the cation effective at the lowest concentration.

6. Valinomycin stimulates the oxidation of Krebs-cycle intermediates at low K^+ concentrations, while nigericin induces a transient inhibition. Monensin has no effect.

INTRODUCTION

In the preceding paper[1] we have shown the important role which K^+ plays in the transport and metabolism of several metabolites in *Azotobacter vinelandii.* Several other investigators[2–6] have stressed the involvement of K^+ in transport and metabolism in different bacteria. The most comprehensive study of K^+ and Na^+ movement in connection with proton production has been made in *Streptococcus faecalis* by Harold *et al.*[7–9].

From most results it cannot be concluded whether K^+ acts intracellularly by stimulating one or more enzymes connected with the metabolism of the substrate studied or by stimulating the transport of substrates. In *Escherichia coli* it has been concluded by Miller *et al.*[3] that the stimulation of the oxidation is paralleled by an increased K^+ uptake. They suggest that K^+ acts inside the cell. Weiden *et al.*[4], however, conclude from their results that in the case of phosphate transport, K^+

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acts in some way extracellularly. Frank and Hopkins[10] report that the transport of glutamate in *E. coli* is stimulated by Na^+.

We are interested in the transport of various Krebs-cycle intermediates in *A. vinelandii* which can be shown to occur via different translocators[11]. Furthermore we are interested in the relationship between transport and metabolism of these di- and tricarboxylic acid anions. As we have shown earlier[11], during succinate oxidation by succinate-grown cells, accumulation of Krebs-cycle intermediates occurs. The question arises whether this uptake of Krebs-cycle intermediates is accompanied by an uptake of cations, to compensate for the negative charges of the Krebs-cycle intermediates. For this reason we have measured the movement of K^+, Na^+ and H^+ across the membrane during metabolism.

METHODS

A. vinelandii (strain ATCC 478) was grown, harvested and washed as described elsewhere[11].

The rate of oxygen uptake was measured polarographically with a Clark electrode in a medium containing 25 mM Tris–HCl, pH 7.6.

Malate dehydrogenase (decarboxylating) (NADP) (EC 1.1.1.40) was measured in an extract of *A. vinelandii*[11] according to Hsu and Lardy[12], in a medium containing 100 mM Tris–HCl, 4 mM $MgCl_2$, 0.2 mM $NADP^+$ and 0.5 mM Tris–L-malate, final pH 7.4.

Protein was determined according to Gornall *et al.*[13].

Measurement of K^+ and Na^+ spectrophotometrically

K^+ and Na^+ were measured spectrophotometrically with a Perkin–Elmer atomic absorption spectrophotometer (model 305).

Measurement of K^+, hydrogen and oxygen with electrodes

Simultaneous measurement of K^+, hydrogen and oxygen was performed with an apparatus as described by Pressman[14], using a sensitive pH-measuring system (Electronic Instruments Ltd), incuding sensitive hydrogen and potassium glass electrodes, 2 pH-measuring units (C33B-2) and Vibron electrometers (33B-2). Oxygen was measured with a Clark-type electrode. The medium contained routinely 1 mM Tris–HCl, 0.1 mM KCl and substrate, final pH 7.6. When the effect of nigericin or valinomycin was tested, 0.4 mM sodium EDTA was added[1].

Special chemicals

Valinomycin, nigericin and monensin were a gift of Eli Lilly and Comp.

RESULTS

Stimulation of the oxidation by cations

Succinate-grown cells of *A. vinelandii* can oxidize Tris–succinate in a Tris–HCl medium. The K^+ concentration in that case is about 10–20 μM derived from the K^+ present in the cell suspension. Addition of 1 mM KCl can stimulate the oxidation more than 3-fold in some cases. After the addition of KCl the new steady-state

TABLE I

THE EFFECT OF CATIONS ON THE OXIDATION OF SUCCINATE BY *A. VINELANDII*

The oxidation was measured as described in Methods, using succinate-grown cells in a medium containing 25 mM Tris–HCl and 6 mM Tris–succinate, final pH 7.6. Cations were added as their chloride salts to a final concentration of 6 mM.

Cation	*Oxidation velocity (μatoms O/min per mg protein)*	*Stimulation (%)*
None	0.47	—
K^+	1.21	157
Na^+	0.75	60
Rb^+	0.69	47
NH_4^+	0.54	15
Li^+	0.52	10
Mg^{2+}	0.48	2
Ca^{2+}	0.47	0

TABLE II

STIMULATION OF THE OXIDATION BY K^+ AND Na^+

Cells were adapted to different substrates as described in ref. 11. The oxidation was measured in a medium containing 25 mM Tris–HCl, 6 mM KCl or NaCl and 6 mM of the substrate indicated, neutralized with Tris, except for pyruvate and lactate, when 12 mM was used. Final pH 7.6.

Cells adapted to	*Substrate*	*Stimulation (%)*	
		+KCl	*+NaCl*
Succinate	Succinate	250	55
	Lactate	200	5
	Pyruvate	285	300
Succinate+2-oxoglutarate	Succinate	150	60
	2-Oxoglutarate	109	70
Citrate	Citrate	95	50

velocity is reached within 1 min. Similar results concerning the stimulation of the oxidation by K^+ have been reported by Miller *et al.*[3] in *E. coli*, although the process in that case was much slower. Also Na^+ and NH_4^+, although in higher concentrations, can stimulate the oxidation in *A. vinelandii*. Table I shows the effect of different cations on the succinate oxidation. Table II shows the effect of K^+ and Na^+ on the oxidation of different substrates.

Although cations increase the maximal oxidation velocity, they do not change the K_m of substrate for oxidation. For instance the K_m for succinate is 80 μM in the presence or absence of K^+.

Fig. 1 shows a plot of the inverse of the cation concentration *versus* the inverse of the percentage stimulation with succinate as a substrate. Defining the reciprocal of the intercept of the graph with the abscissa as the "K_m of activation", we find about 1 mM for K^+ and 10 mM for NH_4^+. The K_m for Na^+ is difficult to determine because there is no linear relationship in the double-reciprocal plot. Whereas these values apply to succinate oxidation, we find a K_m of activation for K^+ of about 100 μM when we study 2-oxoglutarate oxidation.

As can be seen from Table II, the oxidation of all Krebs-cycle intermediates is stimulated by K^+ or Na^+, although to a different degree. Divalent cations have no stimulatory effect except in the case of citrate. Citrate oxidation is stimulated both by Mg^{2+} and Ca^{2+}.

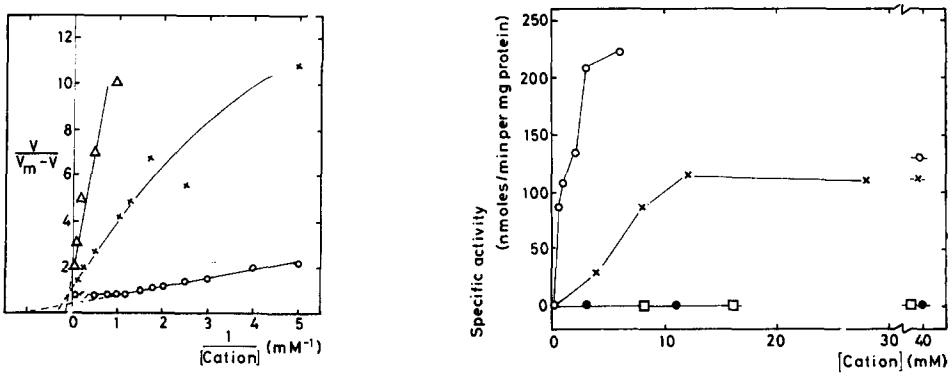


Fig. 1. Plot of the inverse of the stimulation of oxidation *versus* the inverse of the cation concentration. The oxidation was measured as described in Methods, using a medium containing 25 mM Tris–HCl and 6 mM Tris–succinate, pH 7.6; v, oxidation velocity in the absence of cations; V_m, oxidation velocity in the presence of the cation. △—△, NH_4^+; ×—×, Na+; ○—○, K^+.

Fig. 2. Stimulation of "malic" enzyme by cations. "Malic" enzyme was isolated and measured as described in Methods. ×—×, K^+; ○—○, NH_4^+; ●—●, Na^+; □—□, Li^+.

Stimulation of "malic" enzyme

In principle all enzymes participating in the metabolism *via* the Krebs cycle could be stimulated by cations, in this way explaining the stimulatory effect on the oxidation by intact cells. It is known that "malic" enzyme in particular is stimulated by various monovalent cations as for instance reported by Spina *et al.* in *E. coli*[15]. Fig. 2 shows that K^+ and NH_4^+ can stimulate the activity of "malic" enzyme, but Na^+ and Li^+ are unable to do so.

K^+ and Na^+ content of cells

Cells upon harvesting and washing lose K^+ as shown in Table III. The concentration of K^+ is calculated assuming an intracellular water volume of 5 μl/mg protein[11]. As we will show below, the uptake of K^+ is dependent on the amount of K^+ already present intracellularly. The maximal concentration of K^+ that is reached under metabolic conditions is about 40–45 mM. In addition, extensively washed cells contain 5–10 mM Na^+. The cells routinely used in our experiments contain

TABLE III

THE K^+ CONTENT OF CELLS AFTER SEVERAL WASHINGS

Cells (200 mg protein) were harvested and after centrifugation suspended in 100 ml distilled water. This was repeated several times. After resuspension samples were taken and quickly centrifuged in an Eppendorf centrifuge (1 min). K^+ in the supernatant and cell pellet was determined with the atomic absorption spectrophotometer. Concentrations were calculated, assuming an intracellular water volume of 5 μl/mg protein.

Number of washings	$[K^+]$ (*mM*)	
	Intracellularly	*Medium*
1	50	2.5
2	30	0.25
3	21	0.18
4	10	0.12
5	9	0.08
6	10	0.04

20–30 mM K^+ (twice washed) which agrees well with the 40–50 mM KCl needed to stabilize protoplasts[1].

Fig. 3 shows the uptake of K^+ by twice-washed cells with which usually experiments are performed. At low K^+ concentration there is no or almost no uptake above the 30 mM already present. Only at higher external concentration K^+ is taken up during metabolism. In *E. coli* Miller *et al.*[3] report a much larger uptake at low K^+ concentrations. Under anaerobic conditions or without oxidizable substrate, there is no uptake of K^+. Na^+ is not taken up, neither under aerobic nor anaerobic conditions. We showed already in the previous paper[1] that Na^+ is not permeable as

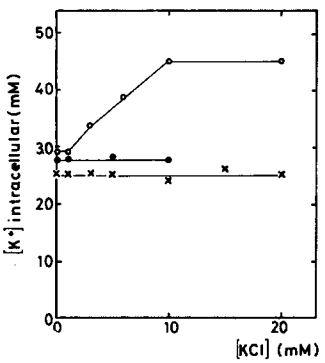


Fig. 3. Uptake of K^+ under aerobic and anaerobic conditions. Succinate-grown cells were incubated in a medium containing 25 mM Tris–HCl, 6 mM Tris–succinate (if present) and the indicated concentration KCl; final protein concentration 2.6 mg/ml; final pH 7.6. O_2 or Ar were bubbled through to keep the solution aerobic and anaerobic, respectively. The reaction was started by adding the cells. After 5 min samples were taken and quickly centrifuged in an Eppendorf centrifuge (1 min). K^+ in the cell pellet was measured with the atomic absorption spectrophotometer. The calculated intracellular concentration was corrected for adhering water containing KCl. ○—○, Tris–succinate *plus* O_2; ●—●, O_2; ×—×, Tris–succinate *plus* Ar.

measured by swelling experiments. It should be emphasized that under these conditions (low K^+ and Na^+) these cations still stimulate the oxidation of Krebs-cycle intermediates.

The movement of K^+ and H^+ during metabolism

In a previous publication[11] we reported that upon oxidation of Krebs-cycle intermediates the medium becomes acid. The same phenomenon was observed by Knowles and Smith[16] during mannitol oxidation by *A. vinelandii*. They suggested that proton extrusion is connected with cation movements, as found by several other authors in various bacteria[17–19]. We have measured the kinetics of the H^+ and K^+ movement during metabolism.

Fig. 4 shows the uptake of K^+ and the production of H^+ extracellularly (or the disappearance of OH^-) during succinate oxidation by succinate-grown cells which were washed extensively. The medium contains 0.1 mM KCl. From Fig. 4 it is clear that K^+ is taken up without a measurable lag after starting the reaction. H^+ production and oxygen consumption, however, show a lag before reaching a steady-state velocity. We have shown earlier[11] that the same lag occurs in the CO_2 production. When the medium becomes anaerobic K^+ uptake stops. Upon a subsequent oxygen pulse K^+ is taken up again till a maximum is reached. Under certain conditions K^+ starts to leak out of the cells upon anaerobiosis. No K^+ uptake occurs anaerobically as found with *E. coli*[20]. However, one should remember that *A. vinelandii* is an obligately aerobic organism.

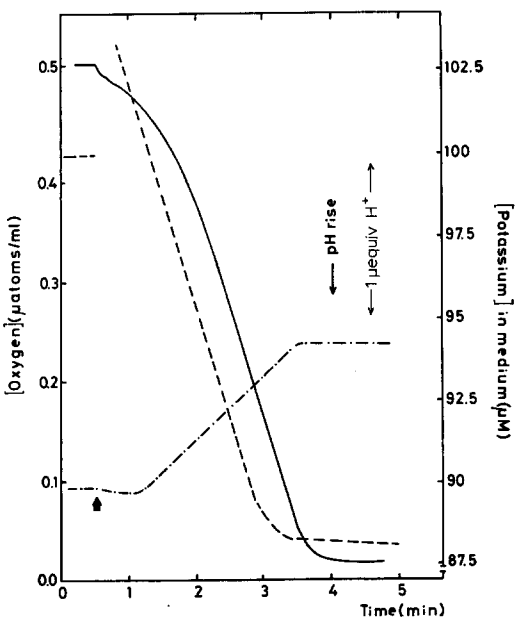


Fig. 4. Movement of K^+ and H^+ during oxidation of succinate. K^+, H^+ and oxygen were measured as described in Methods. The reaction was started by adding 2 mg cell protein (as indicated by the arrow) to a medium containing 1 mM Tris–HCl, 2 mM Tris–succinate and 0.1 mM KCl; final volume 10 ml, final pH 7.6. Succinate-grown cells were 6 times washed. ———, oxygen; – – – –, K^+; —·—, H^+.

TABLE IV

O/H^+ VALUES MEASURED DURING OXIDATION IN *A. VINELANDII*

Cells were adapted to different substrates as described in ref. 11. Oxygen and H^+ were measured as described in Methods. The changes in oxygen and H^+ are expressed in μatoms O per min per mg protein and μequiv H^+ per min per mg protein, respectively. The theoretical values are based on the reaction equations, assuming complete oxidation of the substrate.

Cells adapted to	*Substrate*	*O/H^+*	
		Measured	*Theoretical*
Succinate	Succinate	3.5–4.5	3.5
	Fumarate	3.1	3.0
	L-Malate	3.8	3.0
	Oxaloacetate	2.5	2.5
	Lactate	2.4–3	3.0
	Acetate	3.0–4.5	4.0
	Glucose	2.0	2.0
2-Oxoglutarate	2-Oxoglutarate	2.7	2.67
Citrate	Citrate	2.9–3.6	3.0

From data like those presented in Fig. 4 and similar data with other Krebs-cycle intermediates as substrate, the number of moles H^+ produced per mole of oxygen consumed can be calculated. Table IV collects these O/H^+ values, found with different substrates. For comparison we have included the theoretical O/H^+ values which can be calculated on the assumption that substrates are completely oxidized to CO_2 and water, a condition which is met approximately in *A. vinelandii*[11]. For instance for succinate the O/H^+ value should be 3.5, based on the net reaction equation (at pH 7.6):

$$C_4O_4H_4^{2-} + 7O + H_2O \rightarrow 4HCO_3^- + 2H^+$$

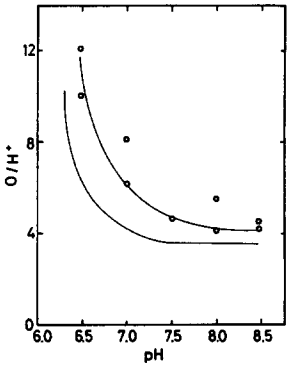
(The pK values of H_2CO_3 at 25 °C are 6.37 and 10.25, respectively[21]).

Fig. 5 shows in addition that indeed the O/H^+ value is dependent on the pH as expected. Because of the decreased dissociation of H_2CO_3 at lower pH we expect an increase of the measured O/H^+ value, as found experimentally.

Uptake of K^+ during metabolism

Although we have shown now that the expulsion of protons is mainly due to the formation of CO_2 which derives from metabolism, it cannot be excluded that possibly a small part of the protons is linked to cation uptake. For this reason we have measured the kinetics and extent of the K^+ movement, assuming that Na^+ is not permeant.

Fig. 6 shows that the net amount of K^+ taken up per mg protein is dependent on the oxidation velocity when succinate is used as a substrate. The amount of K^+ taken up increases with higher oxidation velocity at a fixed pH. Knowles and Smith[16] report that only small amounts of K^+ are taken up in *A. vinelandii* and that the rate of uptake is also low. This is probably due to the substrate they used, because with glucose as a substrate we also find low values both for the rate and the extent.



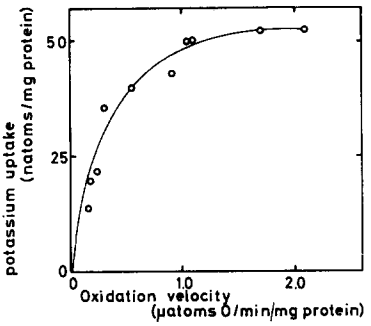
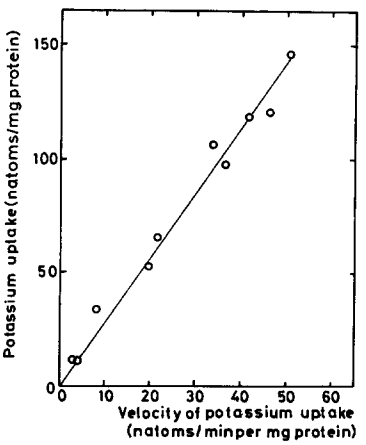


Fig. 5. O/H^+ value as a function of the pH. Oxygen consumption and H^+ production were measured as described in Methods. Succinate-grown cells were oxidizing 6 mM Tris–succinate in a medium containing 25 mM Tris–HCl, at different pH. The theoretical O/H^+ values were calculated as described in the text. ○—○, measured values; ———, theoretical values.

Fig. 6. K^+ uptake as a function of the oxidation velocity. Succinate-grown cells were oxidizing Tris–succinate in a medium containing 0.15 mg protein/ml, 25 mM Tris–HCl and 0.1 mM KCl, final pH 7.6. The oxidation rate was varied by varying the succinate concentration. K^+ uptake was measured with a sensitive K^+ electrode as described in Methods.

Fig. 7 shows that there exists a linear relationship between the amount of K^+ taken up and the velocity of K^+ uptake. In these experiments the change in the oxidation velocity is brought about by changing the substrate concentration. It can be shown (Postma, P.W., unpublished) that the amount of Krebs-cycle intermediates accumulated intracellularly is also dependent on the outside concentration.



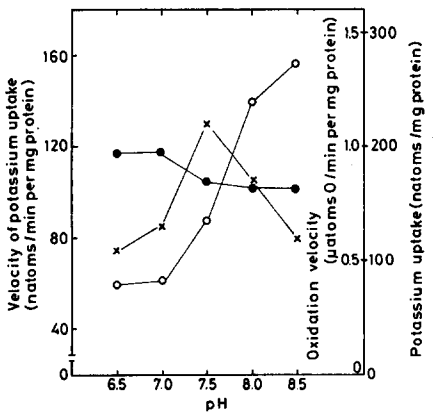


Fig. 7. Relationship between the extent and the velocity of potassium uptake during succinate oxidation. The reaction medium contained 25 mM Tris–HCl, 0.1 mM KCl, varying concentrations of succinate and 0.15 mg protein/ml, final pH 7.6. K^+ uptake was measured as described in Methods.

Fig. 8. Effect of pH on oxidation, extent of K^+ uptake and velocity of K^+ uptake. Succinate-grown cells were oxidizing Tris–succinate in a medium containing 1 mM Tris–HCl, 1 mM Tris–succinate, 0.1 mM KCl and 0.15 mg protein/ml; O_2 and K^+ were measured as described in Methods. ○—○, rate of K^+ uptake; ×—×, rate of oxidation; ●—●, extent of K^+ uptake.

It is difficult to compare directly the amount of K^+ accumulated in different cell preparations with different substrates because the accumulation depends on the K^+ already present intracellularly. However, using cells adapted to different substrates simultaneously, we have measured the uptake of K^+ using different substrates. The final level reached is about the same with different substrates, unless the oxidation rate is very different.

Fig. 8 shows in addition that the linear correlation between the oxidation velocity and the rate of K^+ uptake does not hold if we vary the pH and that the final level of accumulation is independent of the pH.

Because the K^+ electrode becomes insensitive at higher K^+ concentrations we were unable to measure accurately the K^+ uptake at high concentrations. However, it looks as if the K_m for K^+ uptake is lower (<0.2 mM) than the K_m for the stimulation of the succinate oxidation, as reported in Fig. 1.

Effects of valinomycin and nigericin

Not many reports have been published on the effects of nigericin, valinomycin or monensin in bacteria. The most extensive study has been made in *Streptococcus faecalis* by the group of Harold and Baarda (for a review see ref. 22). Pressman[23] reports that valinomycin stimulates the K^+ transport in *Mycobacterium phlei* and *A. vinelandii*, but not in *E. coli*. As we reported in the previous paper[1], valinomycin and nigericin are effective in *A. vinelandii* provided the cells are treated with EDTA. To solve the question whether antibiotics that are known to stimulate the K^+ movement have also an effect on the oxidation, we measured the effect of nigericin and valinomycin on the oxidation velocity and the movement of K^+ and H^+.

Fig. 9A shows that valinomycin stimulates the oxidation of succinate in a medium containing 100 μM KCl. K^+ is taken up rapidly while protons are extruded with a burst. After anaerobiosis the K^+ leaks out again rapidly, in contrast to cells without valinomycin. Pressman[23] does not find this rapid extrusion of K^+, at least in *M. phlei*. The uptake of K^+ amounts to about 20 mM. The question remains, however, whether the stimulation of the oxidation is connected to the increased K^+ uptake in the sense that the K^+ accumulated activates one or more enzymes involved in the oxidation. Experiments show furthermore that the same stimulation by valinomycin is observed with K^+ concentrations ranging from 50 μM to 1 mM, the final oxidation velocity being constant.

To check whether the oxidation always requires a high K^+ concentration inside the cell, we measured the effect of nigericin which is known to catalyze a 1:1 K^+–H^+ exchange[24]. Fig. 9B shows that nigericin induces leakage of K^+ out of the cells, coupled to an uptake of H^+. Calculation shows, however, that more than one K^+ is extruded per H^+ taken up. Temporarily the oxidation is inhibited but after a while returns to its original value. During the recovery no net K^+ is taken up. This result suggests that K^+ is not absolutely required for oxidation. The transient inhibition of the oxidation could be due to a lowering of the internal pH as a result of the H^+ uptake. Measurements with labelled 5,5-dimethyloxazolidinedione-2,4 have shown that in the range of pH 6 to 9 (outside) the inside pH nearly equals the outside pH (Postma, P. W., unpublished), in contrast to findings in *Micrococcus denitrificans*[25]. Fig. 8 shows that the oxidation is very dependent on the pH.

At higher concentrations it was impossible to measure K^+ movement, but on

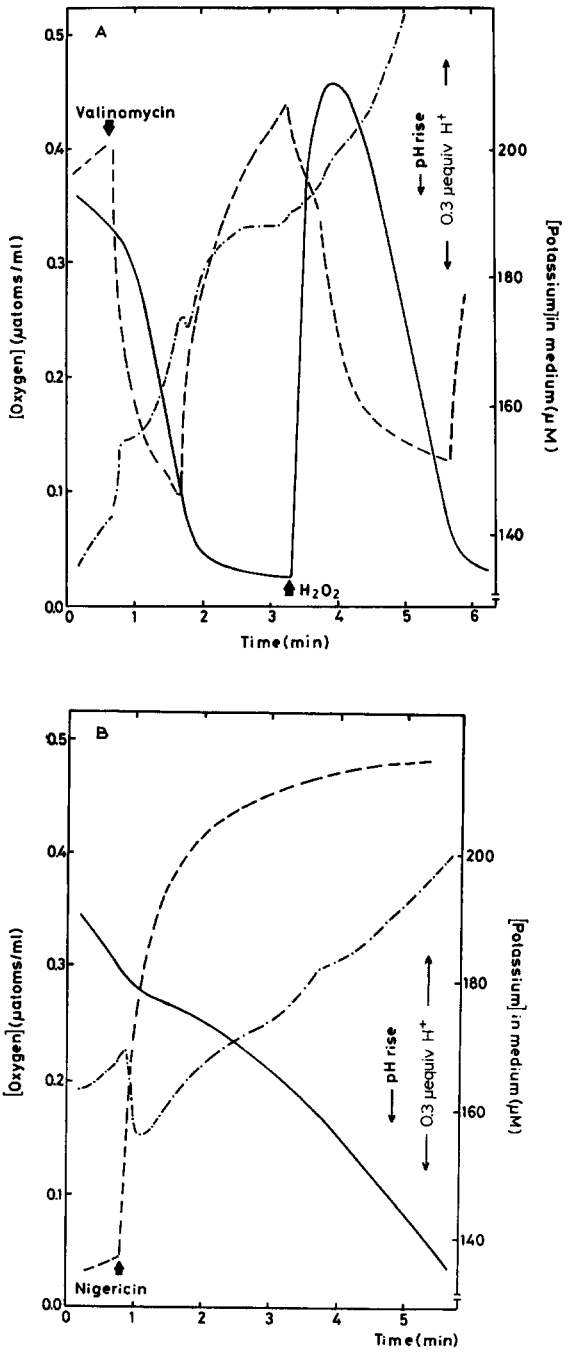


Fig. 9. Effect of nigericin and valinomycin on the oxidation of succinate. Succinate-grown cells (twice washed) were oxidizing Tris–succinate in a medium containing 2 mg protein (A) or 3.5 mg protein (B), 1 mM Tris–HCl, 1 mM Tris–succinate, 0.1 mM KCl and 0.4 mM EDTA, final volume 6 ml, final pH 7.6. In A, 1 μg valinomycin and in B, 1 μg nigericin was added.

the oxidation we see still an effect. Valinomycin is somewhat inhibitory at high K^+ concentration outside, while nigericin still gives a transient inhibition. The combination of valinomycin *plus* nigericin is inhibitory both at low (0.1 mM) and high K^+ concentrations. This is reasonable because the net effect is cyclic cation transport at the expense of energy. Uncouplers like carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole (TTFB) inhibit the oxidation also at concentrations below 1 μM.

Monensin (up to 10 μg/ml) had no effect on the succinate oxidation, whether sodium was present or absent.

DISCUSSION

The results presented in this and the preceding paper[1] show the close connection between anion and cation transport in *A. vinelandii* and the effect of cations on the metabolism. In a variety of microorganisms it has been reported that cations can stimulate uptake or metabolism of sugars[2,26,27], amino acids[2,3,10], Krebs-cycle intermediates[3,6] or inorganic phosphate[4].

Since the metabolism of Krebs-cycle intermediates involves also the transport of these anions across the membrane, at least two explanations can be advanced for the stimulation of the oxidation in *A. vinelandii*. Either cations activate the translocator or cations, once accumulated intracellularly, increase the activity of one or more enzymes involved in oxidation.

Although we cannot exclude definitely the second explanation, we think that in any case cations increase the activity of the translocator. Several reasons can be brought forward. Firstly, when the K^+ concentration in the medium is low ($\leq$1 mM), no net K^+ is taken up (see Fig. 3) although in these twice-washed cells K^+ stimulates the succinate oxidation at least 2-fold.

Secondly, Na^+ also stimulates the oxidation of Krebs-cycle intermediates while both swelling experiments[1] and the direct determination of the sodium content of cells indicate that Na^+ is not taken up.

Thirdly, the oxidation in the presence of nigericin shows clearly that intracellular K^+ is not obligatorily involved in the oxidation of succinate. The transient inhibition of the oxidation can be explained probably by a transient lowering of the internal pH.

Lastly, the maximal stimulation of the oxidation of succinate and 2-oxoglutarate occurs at different extracellular K^+ concentrations, 1 mM and 100 μM, respectively. Considering the fact that succinate and 2-oxoglutarate are oxidized at about the same rate (when K^+ is not limiting) and that K^+ is taken up to the same extent with both substrates, it seems unlikely that K^+ acts intracellularly.

However, some experiments are a little more difficult to reconcile with the concept that K^+ acts only at the outside of the cell. Fig. 9A shows that valinomycin increases the succinate oxidation to a large extent with a concomitant uptake of K^+. In this experiment the extracellular K^+ concentration is 100–200 μM. This does not correlate with the earlier finding that K^+ stimulates maximally when the outside concentration is equal to or greater than 1 mM. It is possible that valinomycin acts by lowering the K_m of the translocator for K^+. We do not know whether K^+ acts directly on the translocator or indirectly by making the membrane more positive

(or less negative) in that way promoting the transport of anions through the membrane.

In mitochondria an effect of cations on the translocators has been reported by Meisner *et al.*[28]. In that case, however, cations change the affinity for the substrates but not the maximal velocity. We find that cations do not change the affinity for Krebs-cycle intermediates but instead increase the maximal velocity.

It is remarkable that valinomycin acts at the low K^+ concentrations used here. Moore and Pressman[29] report a K_m for K^+ of 5 mM in mitochondria, while also in lipid bilayer systems K^+ is needed in the millimolar range[30]. In our system valinomycin acts already with 10 μM K^+. A similar conclusion can be drawn from the data of Bhattacharyya *et al.*[31] in *E. coli.*

The extra K^+ taken up in the presence of valinomycin cannot be retained inside the cell upon anaerobiosis, in contrast to the K^+ normally taken up (compare Figs 4 and 9A).

Although we conclude from our results that cations stimulate extracellularly, it is clear that K^+ can be taken up by the cells establishing a gradient from inside to outside of at least 400 in some cases. We think that K^+ is needed inside the cell to compensate for the electrical charge of Krebs-cycle intermediates and other anions that are accumulated intracellularly during metabolism[11]. We have concluded earlier[1] that an energy-linked K^+ pump must exist in *A. vinelandii* but from our data we cannot decide whether the primary process is proton expulsion followed by a movement of K^+ down the electrochemical gradient or that first K^+ is taken up in an energy requiring way followed by proton expulsion or anion uptake. It should be kept in mind that the protons found outside during metabolism are mainly or only due to the product of the Krebs-cycle oxidation, CO_2. K^+ uptake linked to proton extrusion or Na^+ extrusion has been described in *Streptococcus faecalis*[7,32].

The net amount of K^+ taken up in *A. vinelandii* is small compared to the amount of Krebs-cycle intermediates that moves across the membrane during oxidation. From Fig. 4 and similar data on other substrates it can be calculated that in the case of succinate as substrate about 1 mole of K^+ is taken up per 30 moles O consumed, during the linear part of the K^+ uptake. When the inside K^+ concentration is 40–45 mM, no net K^+ is taken up, but oxidation continues. It is possible that K^+ moves together with anions on the translocator. In that case $KHCO_3$ must diffuse back. This K^+ movement, if present, is different in any case from the proposed K^+ pump because this pump is present both in sucrose-grown cells and cells adapted to different Krebs-cycle intermediates.

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