Osmotic effects on bacterial transport and energetics

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Paracoccus denitrificans suspended in media containing 20–300 mM NaCl swelled progressively as the salt concentration was decreased. The increase in intracellular water volume was accompanied by an enhancement of respiration and a stimulation of the rates of net potassium and α-aminoisobutyric acid accumulation. It is postulated that influx of water and consequent lowering of intracellular solute concentration trigger transport mechanisms which are destined to restore the original ion and metabolite balance. Since a number of transport reactions operate against the electrochemical gradient of their substrates, energy utilization increases. The increased ATP usage and lowering of [ATP] stimulates the activity of the respiratory chain and increases oxygen uptake and energy production.

P. denitrificans *Volume regulation* *Ion transport* *Respiration regulation*

1. INTRODUCTION

Mammalian cells during their life span are exposed to a relatively constant environment whereas bacteria must often adapt to drastic alterations in the external milieu. During the course of studies on the bacterium *Paracoccus denitrificans* we noticed that its metabolic properties were markedly affected by incubation in media of varying concentrations of salts. Here we show that energy parameters, and ion and substrate movements in *P. denitrificans* are sensitive to changes in osmolarity of the suspending medium.

2. MATERIALS AND METHODS

P. denitrificans was grown and harvested as described in [1]. The measurements of respiratory rate, state of reduction of cytochrome *c*, concentrations of ions and metabolites were all carried out as given in [1]. The uptake of α-amino[^{14}C]isobutyric acid (New England Nuclear, Boston, MA; spec. act. 60 mCi/mmol) was measured by following the increase in radioactivity in the cell pellet obtained after the separation of the bacteria from the external medium by rapid centrifugation (Beckman microfuge) through a layer of silicone oil.

3. RESULTS

3.1. *Effect of [NaCl] on intracellular water volume*

P. denitrificans cells suspended in media with increasing [NaCl] shrank progressively which suggests that the bacterium may behave as an osmometer. If this is so, a plot of cell volume against the reciprocal of the osmotic pressure of the medium ($1/\pi$) should yield a straight line according to the Boyle-van't Hoff relationship. As shown in fig.1, cells supspended in [NaCl] between 50 and 200 mM did shrink linearly with a decrease in $1/\pi$. However, at concentrations below 40 mM NaCl, the bacterium remained maximally swollen, which suggests that the cell wall imposes a limiting value on the volume.

3.2. *Effect of [NaCl] on energy parameters in* P. denitrificans

Table 1 summarizes the effect of [NaCl] on energy parameters in *P. denitrificans*. The

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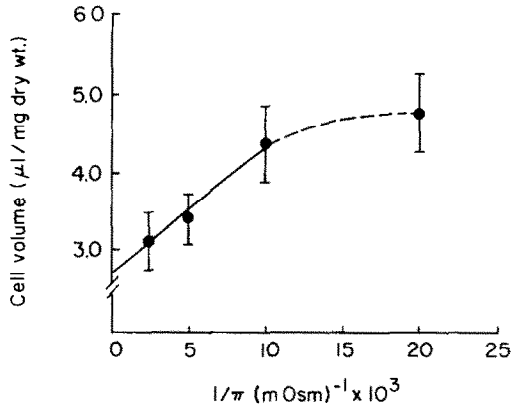

Fig.1. Boyle-van't Hoff's plot of intracellular water volume in *P. denitrificans*. Total water volume of the bacterial pellet was determined from the content of tritiated water whereas the extracellular (trapped) water was calculated from the content of [^{14}C]polyethylene glycol (M_r 4000).

respiratory rate declined progressively with the increase in [NaCl] and this was accompanied by a parallel oxidation of cytochrome *c*. Over the range of salt concentration explored, 20–300 mM, the respiratory rate decreased by 2.65-fold and reduction of cytochrome *c*, by 2.6-fold. The [ATP]/[ADP] · [P_i] was relatively stable at a value of 1400–1600 m^{-1} in 20–200 mM NaCl but declined to about 300 M^{-1} in 300 mM NaCl. Addition of 5 mM potassium chloride caused stimulation of respiration in all media (not shown). In 20–200 mM NaCl the final stimulated rate reached the same value of 28 ± 2 nmol/min per mg dry wt of cells, irrespective of salt concentration. This enhancement of respiratory activity occurred in concert with increase in reduction of cytochrome *c* to about 27–29%. There was a small decline in the [ATP]/[ADP] · [P_i] which was statistically not significant. By contrast, in 300 mM NaCl the maximal respiratory rate was only 19.2 ± 2 nmol/min per mg dry wt and cytochrome *c* became only slightly more reduced (to about 9%).

3.3. *Effect of [NaCl] on ion and substrate transport*

The effect of osmolarity on ion transport was measured by following the amount of potassium taken up by cells suspended in media containing 20, 100 and 200 mM NaCl. Fig.2 shows that the rates of net potassium uptake were fastest in 20 mM NaCl and decreased progressively with increasing osmolarity of the medium. It can also be seen that potassium concentrations inside the cells in the absence of added K were between 60 and 70 mM regardless of osmolarity. After addition of 5 mM K^+ the intracellular concentrations of this cation rapidly increased in all media: at steady state, cells suspended in 20 mM NaCl increased their internal potassium content of 108 ± 12 mM (mean ± SD; $n = 3$) and those in 100 mM NaCl to about 120 ± 21 mM (mean ± SD; $n = 3$). Since the

Table 1

Effect of [NaCl] on energy parameters in *P. denitrificans*

[NaCl] (mM)	Respiratory rate (nmol/min per mg dry wt)	Cytochrome c (% red)	[ATP] (mM)	[ADP] (mM)	[P_i] (mM)
20	14.45 ± 2.3	21.6 ± 2.1	1.48 ± 0.06	0.23 ± 0.05	4.6 ± 0.3
50	11.56 ± 1.8	n.d.	2.18 ± 0.2	0.39 ± 0.3	3.4 ± 0.7
100	8.28 ± 0.9	15.6 ± 2.7	2.63 ± 0.09	0.40 ± 0.1	3.2 ± 0.4
200	6.74 ± 1.2	11.5 ± 1.8	2.48 ± 0.1	0.45 ± 0.03	3.4 ± 0.3
300	5.39 ± 0.7	8.3 ± 1.2	1.36 ± 0.3	0.56 ± 0.06	7.5 ± 0.9

P. denitrificans cells were washed and suspended in media containing appropriate concentrations of NaCl and 10 mM Tris-Hepes, pH 7.3. Incubations were carried out at 3–4 mg dry wt cells/ml with 10 mM glucose for 15 min at 25°C. At the end of the incubation, aliquots were removed for the measurement of respiratory rate and reduction of cytochrome *c*. The remaining portions were quenched with cold perchloric acid (6% final acid concentration) for the determination of ATP, ADP and inorganic phosphate. Values are means ± SD for 3–5 experiments. n.d., not determined

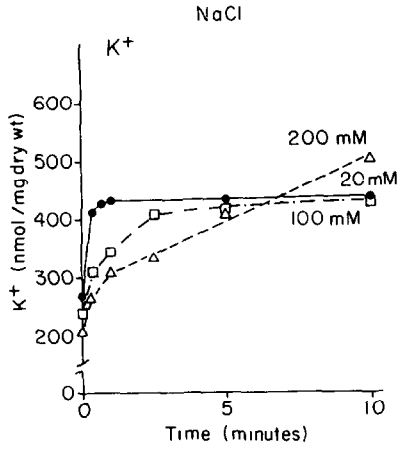

Fig.2. Potassium uptake by *P. denitrificans* in media of different osmolarity. *P. denitrificans* was suspended at about 3–4 mg dry wt/ml in the appropriate media and incubated for 10 min at 25°C with 10 mM glucose. Potassium chloride (5 mM) was added at time '0', aliquots were taken at the intervals indicated and rapidly centrifuged through a layer of silicone oil. Potassium concentration in the pellet was measured by atomic absorption.

concentration of potassium inside the cell was much higher than that in the external environment, the uptake occurred against a large concentration difference and required metabolic energy (note the increase in respiration described above), as reported previously for this [1] and other [2–12] miroorganisms.

The rates of substrate uptake were evaluated by measuring accumulation of α-aminoisobutyric acid, a non-metabolizable amino acid analogue. Table 2 shows that the highest rate was observed in 20 mM NaCl and this was stimulated by less than 2-fold by the addition of 5 mM KCl. In 100 mM NaCl, the rate of amino acid uptake was 10-fold lower than that in 20 mM salt but was stimulated to a much greater extent by the addition of potassium: in the presence of this cation the velocities in the 2 media differed by a factor of less than 2. In solutions of higher osmolarity, 200–300 mM NaCl, the rates of amino acid accumulation in the absence of added potassium were negligible but they were also enhanced by addition of this cation. The enhancement exerted by K^+ was much larger in 200 mM NaCl than in 300 mM salt.

Table 2

Effect of [NaCl] on the rate of α-aminoisobutyrate uptake by *P. denitrificans*

[NaCl] (mM)	α-Aminoisobutyrate uptake (nmol/min per mg dry wt)	
	$-K^+$	$+5$ mM K^+
20	0.47 ± 0.10	0.70 ± 0.15
100	0.04 ± 0.01	0.43 ± 0.09
200	<0.001	0.25 ± 0.04
300	<0.001	0.08 ± 0.01

P. denitrificans cells were washed and suspended as described in the legend to table 1. After 10 min preincubation, uptake studies were initiated by the addition of 10 μM radioactive α-aminoisobutyric acid. Samples were withdrawn at 30, 60, 90 and 120 s and rapidly centrifuged through a layer of silicone oil. Rates were calculated from the amount of radioactivity appearing in the pellet. Values are means ± SD for 3–4 experiments

4. DISCUSSION

The results in this paper contain several interesting points. The first concerns the regulation of intracellular water volume in a prokaryote, *P. denitrificans*. Our studies show that this bacterium is sensitive to changes in tonicity of the external environment and in a certain range of salt concentration complies with Boyle-van't Hoff's law. For the conditions we have investigated the intracellular water space changed by a factor of 2, a relatively large value. This observation was somewhat unexpected because it has generally been assumed that bacteria surrounded by the relatively rigid cell wall are insensitive to changes in tonicity and that osmotic responses are a property of bacterial protoplasts [13]. However, it is worth pointing out that the presence of cell wall does impose a limiting value on volume expansion and hence prevents osmotic lysis and consequent death to the cell.

Although we do not know whether the behavior shown here for *P. denitrificans* is typical of all bacteria, a search through the literature shows that measurements of intracellular water volume in various microorganisms yielded values between 1 and 4.0 μl/mg dry wt, i.e., within the range reported here [2, 14–16]. However most investigators have established the size of the in-

tracellular water compartment for one specific set of conditions and used this value throughout the course of their studies [14–16] or employed values reported by other authors although their own experimental conditions may or may not be the same [17].

The second point concerns the nature of metabolic readjustments which accompany alterations in osmolarity of the external environment. We have shown that a decrease in salt concentration is followed by an enhancement of the rate of ATP synthesis, as manifested in the marked increase in respriatory activity. Since living cells are steady-state systems, this must reflect an augmented demand for metabolic currency. The question then arises as to the nature of the energy-consuming events that are stimulated in media of low osmolarity. It is well-established that bacteria [10–12], like other cells, require certain concentrations of intracellular ions, especially cations such as potassium, for maintenance of their normal physiological functions. Transport of many of these ions occurs against their electrochemical gradient and hence consumes metabolic energy. Our observations suggest that influx of water (which permeates membranes readily) and consequent lowering of intracellular solute concentration triggers mechanism(s) that try to restore the original ion balance. Since ATP is utilized in such reactions, the outcome of this situation is a decrease in the level of ATP and consequent lowering of the $[ATP]/[ADP] \cdot [P_i]$. Although this stimulates and maintains respiration at a higher level, as decrease in cellular [ATP] may be of disadvantage because it might limit activities of reactions which use ATP as the substrate. However, a lowering of the phosphorylation state of the adenine nucleotides can also activate various dehydrogenases and increase substrate influx into the respiratory chain. The consequent reduction of cytochrome *c* allows respiration to increase and restore the original $[ATP]/ADP] \cdot [P_i]$. The final outcome of this chain of events is stimulation of respiration at a markedly more reduced level of cytochrome *c* and relatively unaffected $[ATP]/[ADP] \cdot [P_i]$. The results shown here are consistent with these suggestions. However, when the rate of substrate uptake cannot be activated rapidly enough, stimulation of respiration will occur almost exclusively through a decline in the phosphorylation state of the adenine nucleotides, a situation which occurs in 300 mM NaCl.

In conclusion, bacteria respond very sensitively to changes in external environment and possess mechanisms which couple alterations of ion and substrate movements to cellular energy production. It is interesting to note that the type of regulation of cellular ATP synthesis outlined here for *P. denitrificans* operates also in a number of mammalian cells and tissues [18]. Thus it appears that the same basic control mechanisms apply equally to prokaryotes and multicellular eukaryotes.

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REFERENCES

[1] Erecińska, M., Deutsch, C.J. and Davis, J.S. (1981) J. Biol. Chem. 256, 278–284.
[2] Schultz, S.G., Epstein, W. and Solomon, A.K. (1963) J. Gen. Physiol. 49, 221–234.
[3] Rhoads, D.B., Waters, F.B. and Epstein, W. (1976) J. Gen. Physiol. 67, 325–341.
[4] Padan, E., Zilberstein, D., and Rotenberg, H. (1976) Eur. J. Biochem. 63, 533–541.
[5] Kashket, E.R. and Barker, S.L. (1977) J. Bacteriol. 130, 1017–1023.
[6] Bakker, E.P. and Harold, F.M. (1980) J. Biol. Chem. 255, 433–440.
[7] Kashket, E.R., Blanchard, A.G. and Metzger, W.C. (1980) J. Bacteriol. 143, 128–134.
[8] Brey, R.N., Rosen, B.P. and Sorensen, E.N. (1980) J. Biol. Chem. 255, 39–44.
[9] Plack, R.H., jr and Rosen, B.P. (1980) J. Biol. Chem. 255, 3824–3825.
[10] Harold, F.M. (1977) Curr. Top. Bioenerg. 6, 83–149.
[11] West, I.C. (1980) Biochim. Biophys. Acta 604, 91–126,
[12] Rosen, B.P. (ed.) (1978) Bacterial Transport, Marcel Dekker, New York.
[13] Rothstein, A. (1968) in: Microbial Protoplasts, Sheroplasts and L-Forms (Guze, L.B. ed.) pp. 174–185. Williams and Wilkins, Baltimore.

[14] Freidberg, I. and Kaback, H.R. (1980) J. Bacteriol. 142, 651-658.
[15] Snyder, M.A., Stock, J.B. and Koshland, D.W. (1981) J. Mol. Biol. 149, 241-257.
[16] Heffner, D.L. and Harold, F.M. (1980) J. Biol. Chem. 255, 11396-11402.
[17] Repaske, D.R. and Adler, J. (1981) J. Bacteriol. 145, 1196-1208.
[18] Erecińska, M. and Wilson, D.F. (1982) J. Membrane Biol. 70, 1-14.