JOURNAL OF BACTERIOLOGY, Jan. 1990, p. 149–154
0021-9193/90/010149-06$02.00/0

Vol. 172, No. 1

Osmoregulation in *Rhodobacter sphaeroides*

TJAKKO ABEE, RONALD PALMEN,† KLAAS J. HELLINGWERF,† AND WIL N. KONINGS*
Department of Microbiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

Received 19 June 1989/Accepted 27 September 1989

Betaine (*N,N,N*-trimethylglycine) functioned most effectively as an osmoprotectant in osmotically stressed *Rhodobacter sphaeroides* cells during aerobic growth in the dark and during anaerobic growth in the light. The presence of the amino acids L-glutamate, L-alanine, or L-proline in the growth medium did not result in a significant increase in the growth rate at increased osmotic strengths. The addition of choline to the medium stimulated growth at increased osmolarities but only under aerobic conditions. Under these conditions choline was converted via an oxygen-dependent pathway to betaine, which was not further metabolized. The initial rates of choline uptake by cells grown in media with low and high osmolarities were measured over a wide range of concentrations (1.9 μM to 2.0 mM). Only one kinetically distinguishable choline transport system could be detected. K_t values of 2.4 and 3.0 μM and maximal rates of choline uptake (V_{max}) of 5.4 and 4.2 nmol of choline/min · mg of protein were found in cells grown in the minimal medium without or with 0.3 M NaCl, respectively. Choline transport was not inhibited by a 25-fold excess of L-proline or betaine. Only one kinetically distinguishable betaine transport system was found in cells grown in the low-osmolarity minimal medium as well as in a high-osmolarity medium containing 0.3 M NaCl. In cells grown and assayed in the absence of NaCl, betaine transport occurred with a K_t of 15.1 μM and a V_{max} of 3.2 nmol/min · mg of protein, whereas in cells that were grown and assayed in the presence of 0.3 M NaCl, the corresponding values were 18.2 μM and 9.2 nmol of betaine/min · mg of protein. This system was also able to transport L-proline, but with a lower affinity than that for betaine. The addition of choline or betaine to the growth medium did not result in the induction of additional transport systems.

Adaptation of bacteria to fluctuations in the osmolarity of their surroundings is crucial for their survival. In order to grow, cells must maintain positive turgor, which is an outward pressure resulting from a cytoplasmic osmolarity which exceeds that of the extracellular milieu (9, 13). In *Escherichia coli* osmoregulation and -adaptation have been studied in detail (9, 12). When *E. coli* is exposed to a sudden increase in external osmolarity, dehydration of the cells occurs, which results in an inhibition of growth (2, 19). The bacteria can respond, however, to this osmotic shock by accumulating osmotically active solutes and resume growth when the internal osmotic pressure is appropriately raised (9, 13). The primary response to an increase in osmolarity is the uptake of K^+ ions via the constitutive Trk system, the osmotically regulated Kdp system, or both (6). Although turgor pressure can be restored, growth rates usually remain low because of the inhibition of intracellular enzymes by the high cytoplasmic ionic strength (6, 9). If possible, *E. coli* therefore accumulates so-called compatible solutes to replace K^+. A compatible solute is functionally defined as a compound which can be accumulated to high intracellular concentrations with minimal damage to normal metabolism or enzyme function (9). These osmoprotectants include small organic molecules, such as the amino acids glutamate and proline; betaines; and sugars, such as trehalose (5, 7, 9, 12).

Betaine is accumulated under osmotic stress in widely different organisms such as chemoheterotrophic bacteria (9), halotolerant photosynthetic bacteria, marine animals, and halophilic plants (13). In *E. coli* betaine and its precursor choline have only an osmoregulatory role. These compounds cannot serve as a carbon or nitrogen source, and the expression and activities of the transport systems for betaine and choline are modulated by the osmotic strength of the medium (15, 21).

Until now, nothing has been known about the response of the phototrophic bacterium *Rhodobacter sphaeroides* to increased medium osmolarity. We studied this response and present evidence in this report that betaine is the most effective osmoprotectant in *R. sphaeroides* under osmotic stress during aerobic growth in the dark or during anaerobic growth in the light. Choline also stimulated growth at increased osmolarities, but only under aerobic conditions.

MATERIALS AND METHODS

Culture conditions. *R. sphaeroides* 2.4.1 was grown aerobically in the dark or anaerobically in the light at 30°C in a mineral medium at pH 7.0 containing $MgSO_4 \cdot 7H_2O$ (0.5 g/liter); NH_4Cl (0.4 g/liter); $CaCl_2 \cdot 2H_2O$ (0.02 g/liter); Vishniac trace elements (1 ml/liter); potassium phosphate (pH 7.0; 15 mM); and a vitamin solution (1 ml/liter) containing thiamine hydrochloride (0.5 g/liter), nicotinic acid (1.0 g/liter), and biotin (0.01 g/liter). Sodium DL-lactate (20 mM) was added as a carbon source, an energy source, or both. Media with elevated osmotic strengths were attained by adding KCl or NaCl at the concentrations indicated where appropriate, and the pH was adjusted to 7.0 with KOH or NaOH when necessary. Choline, L-proline, L-alanine, L-glutamate, and betaine (*N,N,N*-trimethylglycine) were added to final concentrations of 1 mM from stock solutions which were sterilized by filtration. Anaerobic growth in the light took place in rubber-stoppered screw-cap tubes which were placed in a water-filled thermostatted glass container. Light was supplied by six 150-W light bulbs. Growth was measured turbidometrically at an optical density of 660 nm. Cells for the inoculum were grown in the minimal medium with no additions.

* Corresponding author.
† Present address: Department of Microbiology, University of Amsterdam, 1018 WS Amsterdam, The Netherlands.

Transport assays. Transport studies in cells were performed at 30°C as described previously (1). Cells were harvested during logarithmic growth, washed twice in the desired buffer in the absence or presence of 50 μg of chloramphenicol per ml, and finally, suspended to a protein concentration of approximately 10 mg/ml. Cells were used immediately or stored on ice and used within 4 h. Experimental conditions are further specified in the legends to Fig. 1 through 5 or in the text.

Measurement of amino acid concentrations. Cells were grown aerobically in the dark in the minimal lactate medium or in media with increased osmolarities, by adding KCl or NaCl. Where indicated, amino acids were added to final concentrations of 1 mM. At the end of logarithmic growth, cells were separated from the medium by silicon oil centrifugation. The amino acids were dansylated and separated by reversed-phase high-performance liquid chromatography as described by Poolman et al. (18). The amino acid concentrations showed less than 5% variation in duplicate experiments.

Fate of intracellular ^{14}C-labeled choline and betaine. Cells were grown in minimal lactate medium or in minimal medium supplemented with 0.3 M NaCl with or without 2 mM choline or betaine. Cells were harvested at an optical density at 660 nm of 0.6, washed twice, and suspended to a final protein concentration of approximately 10 mg/ml in 20 mM potassium phosphate buffer (pH 7.0) containing 20 mM sodium DL-lactate, 5 mM $MgSO_4$, and 50 μg of chloramphenicol per ml supplemented with 0.3 M NaCl when cells were grown in the presence of high concentrations of salt (0.3 M NaCl). Uptake experiments were performed in the buffers described above (1 mg of protein per ml) under aerobic growth conditions in the dark at 30°C, with ^{14}C-labeled choline and betaine being present at 114 and 118 μM, respectively. After 45 min, samples of 1 ml were taken and cells were rapidly separated from the external medium by silicon oil centrifugation. The perchloric acid-EDTA extracts were neutralized with an equal amount of 1 N KOH to $KHCO_3$, and ^{14}C-labeled compounds were analyzed by thin-layer chromatography and autoradiography as described by Speed and Richardson (20).

Analytical procedures. Protein was determined by the method of Lowry et al. (14) by using bovine serum albumin as a standard.

Calculations. The internal volumes of cells grown in the mineral lactate medium and in media with increased osmolarities were determined with [^{14}C]taurine and ^3H_2O as radioactive markers of the extracellular volume, including the periplasmic space, and the total volume, respectively.

Materials. [U-^{14}C]taurine (4.26 TBq/mol), ^3H_2O (37 GBq/mol), L-[U-^{14}C]proline (10.78 TBq/mol), [*methyl*-^{14}C]choline (2.08 TBq/mol), and [*methyl*-^{14}C]betaine (0.29 TBq/mol) were obtained from the Radiochemical Centre (Amersham, United Kingdom). All other chemicals were of reagent grade and were obtained from commercial sources.

RESULTS

Effect of increased osmolarity on growth and intracellular amino acid pools. To analyze the response to increased medium osmolarity, *R. sphaeroides* was grown in a low-osmolarity minimal medium with DL-lactate as a carbon source, an energy source, or both. The specific growth rate under aerobic conditions in the dark decreased from 0.19/h in the absence of NaCl to 0.03/h in the presence of 0.8 M NaCl (data not shown). A similar inhibition of growth at increased NaCl concentrations was observed under anaerobic conditions in the light. Under both conditions inhibition of growth was also observed when the medium osmolarity was increased by the addition of KCl or sucrose.

The intracellular glutamate concentration in cells grown aerobically in the dark in minimal medium was found to be 54.9 mM. The addition of 0.1 and 0.2 M NaCl resulted in a decrease in the specific growth rate to 0.15 and 0.13/h, respectively. The intracellular glutamate concentration increased to 99.1 mM in the presence of 0.1 M NaCl, while in the presence of 0.2 M NaCl the internal concentration decreased to 32.9 mM. No significant changes in the intracellular glutamate concentrations were observed when 1 mM glutamate was added to the medium (data not shown). Other amino acids were not present at high intracellular concentrations (i.e., concentrations did not exceed 2 mM), and these concentrations did not change upon an increase of the medium osmolarity.

The intracellular concentration of proline increased at a high medium osmolarity only when proline was added to the medium. In the presence of 1 mM external proline, the internal concentration in cells during growth in the minimal medium was approximately 0.5 to 0.8 mM. The internal proline concentration increased to 84.9 and 116.4 mM in the presence of 0.1 and 0.2 M NaCl, respectively (data not shown). The specific growth rate increased at a high medium osmolarity (0.2 M NaCl) from 0.13 to 0.16/h in the presence of proline. The specific growth rate in minimal lactate medium supplemented with 1 mM L-proline was significantly higher (0.22/h), which indicates that proline is not a very efficient osmoprotectant in *R. sphaeroides*.

Osmoprotection by choline and betaine. Under aerobic growth conditions in the dark and in the presence of 0.4 M NaCl, the addition of choline or betaine (final concentration, 1 mM) increased the specific growth rate from 0.11 to 0.19/h (Fig. 1A), which was close to the growth rate in the absence of NaCl (0.20/h). Under anaerobic conditions in the light, betaine also increased the specific growth rate in the presence of 0.4 M NaCl from 0.11 to 0.23/h, whereas the addition of choline had hardly any effect on the growth rate (0.12/h; Fig. 1B).

Effects of osmotic strength on choline transport. Cells of *R. sphaeroides* grown aerobically in the dark in the minimal lactate medium and then washed and suspended in a low-osmolarity buffer were able to transport choline at a high rate (4.8 nmol/min · mg of protein) (Fig. 2). Upon the transfer of the cells from the minimal medium without NaCl to a medium with 0.3 M NaCl (upshock), choline uptake decreased to 0.5 nmol/min · mg of protein. A gradual increase in the uptake rate was observed during the first 3 h after upshock (data not shown). This increase in the rate of choline uptake was only slightly inhibited by 50 μg of chloramphenicol per ml, which indicates that the increase in transport activity was not due to de novo protein synthesis.

R. sphaeroides grown in the minimal lactate medium with an elevated osmotic strength (0.3 M NaCl) showed a significant rate of choline uptake (2.6 nmol/min · mg of protein) when the osmolarity was high (0.3 M NaCl). In the low-osmolarity minimal medium without NaCl (downshock), the initial uptake rate was even higher (3.6 nmol/min · mg of protein).

Kinetics of choline transport. The initial rates of choline uptake over a wide concentration range (1.9 μM to 2.0 mM) showed saturation kinetics in cells grown in low- and high-osmolarity media. Eadie-Hofstee plots (Fig. 3) indicated only one kinetically distinguishable choline transport sys-

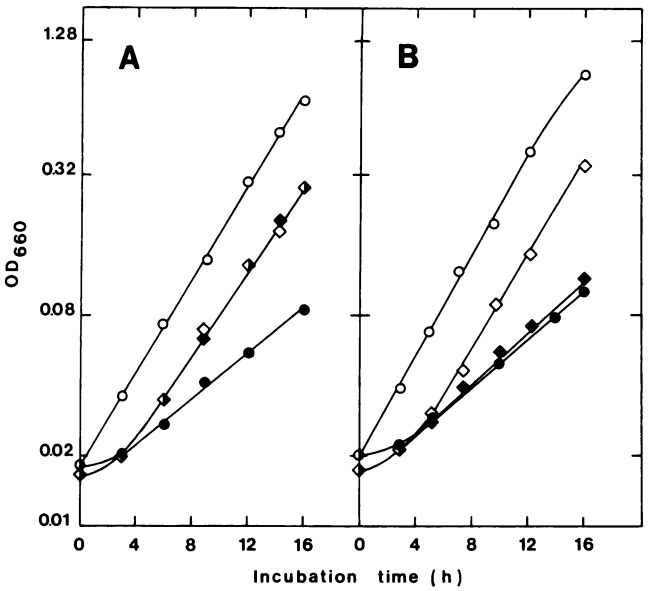

FIG. 1. Effect of choline and betaine on *R. sphaeroides* 2.4.1 during aerobic growth in the dark (A) and during anaerobic growth in the light (B) in minimal lactate medium with 0.4 M NaCl. Symbols: ○, minimal lactate medium; ●, minimal lactate medium with 0.4 M NaCl without additions; ◆, minimal lactate medium with 0.4 M NaCl and 1 mM choline ◇, minimal lactate medium with 0.4 M NaCl and 1 mM betaine. OD_{660}, Optical density at 660 nm.

tem. K_t values of 2.4 and 3.0 μM and maximal rates of choline uptake (V_{max}) of 5.4 and 4.2 nmol of choline/min · mg protein were found in cells grown in minimal medium without or with 0.3 M NaCl, respectively. Cells grown in the presence of 1 mM choline showed the same kinetic values,

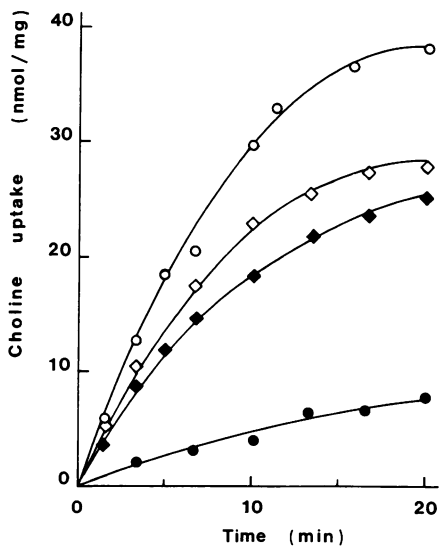

FIG. 2. Effect of osmotic strength on choline uptake in *R. sphaeroides* 2.4.1. Cultures were grown aerobically in the dark in minimal lactate medium without (○, ●) and with (◇, ◆) 0.3 M NaCl. Uptake was assayed in minimal lactate medium containing 50 μg of chloramphenicol per ml without (open symbols) and with (closed symbols) 0.3 M NaCl. Uptake of [^{14}C] choline (final concentration, 114 μM) was initiated after 5 min of preincubation under aerobic conditions in the dark.

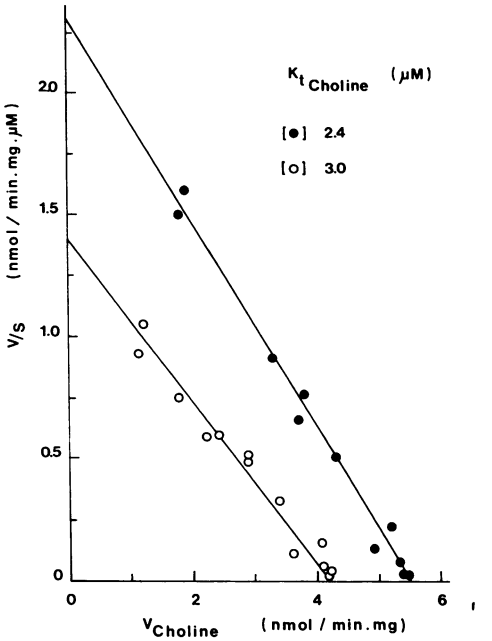

FIG. 3. Kinetic parameters of choline transport in *R. sphaeroides* 2.4.1 grown in the absence and presence of osmotic stress. Uptake was studied in cells that were grown, washed, and suspended in minimal lactate medium without (●) and with (○) 0.3 M NaCl in the presence of 50 μg of chloramphenicol per ml. After 5 min of preenergization, [*methyl*-^{14}C]choline (concentration range, 1.9 μM to 2 mM) was added. Initial rates of uptake were measured in duplicate between 5 and 30 s. The data are presented in the form of Eadie-Hofstee plots.

indicating that under these conditions no additional transport system was induced (data not shown).

The transport system was not inhibited by a 25-fold molar excess of L-proline or betaine (data not shown).

Fate of accumulated choline. Cell extracts were analyzed by thin-layer chromatography after uptake of [^{14}C]choline. In cells grown in minimal lactate medium without NaCl, both choline and betaine were found. This indicates that choline is partially converted to betaine even under conditions of low osmotic strength. In cells grown in minimal lactate medium supplemented with 0.3 M NaCl, very little choline and much more betaine could be detected, indicating that, under conditions of high osmotic strength, most of the choline is converted to betaine. Furthermore, these results suggest that choline metabolism is activated by osmotic stress in *R. sphaeroides* under these conditions.

Autoradiograms of two-dimensional thin-layer chromatographs of cell extracts after uptake of [^{14}C]betaine showed one spot in cells grown in minimal lactate medium with and without 0.3 M NaCl (data not shown). This observation indicated that betaine is not metabolized in *R. sphaeroides* under these conditions.

Effects of osmotic strength on betaine transport. Betaine uptake was observed in cells of *R. sphaeroides* that were grown aerobically in the dark in minimal lactate medium without salt (Fig. 4). The initial betaine uptake rate was 2.4 nmol/min · mg of protein in cells that were washed and suspended in a low-osmolarity medium. The rate of uptake increased to 8.0 nmol/min · mg of protein, and betaine was accumulated to 132.4 mM, when the cells were washed and suspended in a high-osmolarity medium (0.3 M NaCl).

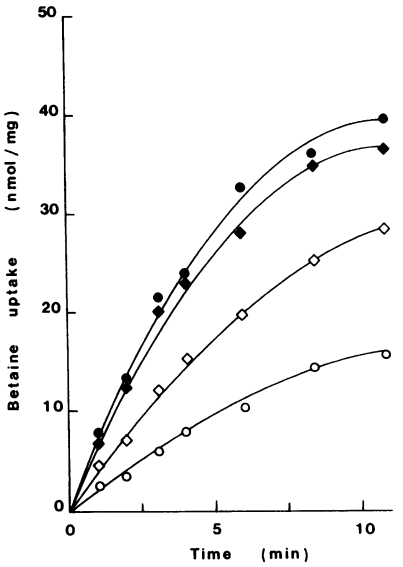

FIG. 4. Effect of osmotic stress on betaine uptake in *R. sphaeroides* 2.4.1 grown aerobically in the dark. Cultures were grown in minimal lactate medium without (○, ●) and with (◇, ◆) 0.3 M NaCl. Uptake was assayed aerobically in the dark in minimal lactate medium without (open symbols) and with (closed symbols) 0.3 M NaCl supplemented with 50 μg of chloramphenicol per ml. After 5 min of preenergization, [*methyl*-^{14}C]betaine (final concentration, 118 μM) was added.

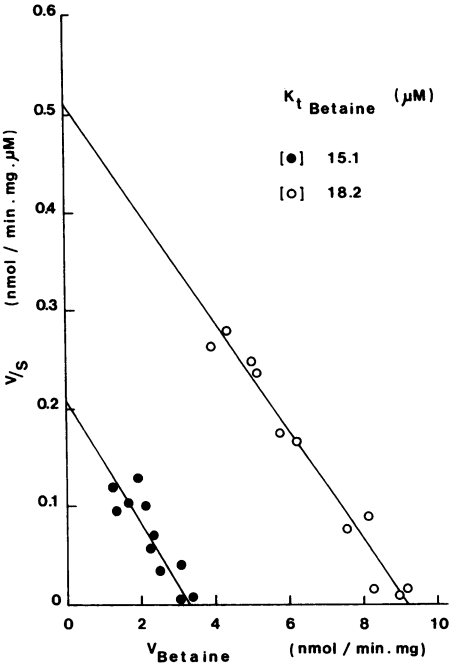

FIG. 5. Kinetic parameters of betaine transport in *R. sphaeroides* 2.4.1 grown in the absence and presence of osmotic stress. Uptake was studied in cells that were grown, washed, and suspended in minimal lactate medium without (●) and with (○) 0.3 M NaCl in the presence of 50 μg of chloramphenicol per ml. After 5 min of preenergization, [*methyl*-^{14}C]betaine (concentration range, 10 μM to 2 mM) was added. Initial rates of uptake were measured in duplicate between 5 and 30 s. The data are presented in the form of Eadie-Hofstee plots.

In cells that were grown in medium with an elevated osmotic strength (0.3 M NaCl), a high uptake rate of betaine (7.2 nmol/min · mg of protein) was also observed in the presence of 0.3 M NaCl. The initial rate of betaine uptake decreased to 2.4 nmol/min · mg of protein in these cells in the absence of NaCl. Immediate stimulation of betaine transport by NaCl in cells grown at low osmolarities was also found in the presence of 50 μg of chloramphenicol per ml.

Kinetics and substrate specificity of betaine transport. The initial rates of betaine uptake in cells grown in the minimal lactate medium with or without 0.3 M NaCl were determined over a wide range of concentrations (10 μM to 2.0 mM). Eadie-Hofstee plots of the data (Fig. 5) showed only one kinetically distinguishable betaine transport system in cells grown in low-osmolarity minimal medium as well as in high-osmolarity medium. A K_t of 15.1 μM and a V_{max} of 3.2 nmol/min · mg of protein for betaine transport was determined in cells that were grown and assayed in the absence of NaCl, whereas in cells that were grown and assayed in the presence of 0.3 M NaCl, the values were 18.2 μM and 9.2 nmol of betaine per min · mg of protein, respectively. The same betaine transport system thus seems to be operative in low- and high-osmolarity-grown cells, and no additional transport systems were induced when 1 mM betaine was present during growth (data not shown).

Transport of betaine was partially inhibited (approximately 33%) by a 25-fold excess of L-proline but not at all by a 25-fold excess of choline, indicating that the betaine transport system is also able to transport L-proline, but with a lower affinity than that for betaine. This conclusion is supported by the observation that uptake of L-proline is more effectively inhibited (approximately 70%) by betaine, especially at high L-proline concentrations (data not shown).

DISCUSSION

This study presents evidence that betaine most effectively protects *R. sphaeroides* against osmotic stress. Growth of *R. sphaeroides* was inhibited by a high osmolarity of the medium. This inhibition was most likely caused by dehydration of the cells, which resulted in a high intracellular ionic strength, as has been described for *E. coli* (2, 19). In a minimal medium with lactate as the sole carbon or energy source and ammonia as a nitrogen source, glutamate appeared to be the only amino acid whose intracellular concentration increased to some extent with the osmolarity of the medium. Variations in the internal glutamate concentration occurred in the absence of exogenously added glutamate, which indicated that these changes are due to metabolism. Similar observations have been made in *E. coli* and other bacteria. Glutamate dehydrogenase has been shown to be activated under these conditions, which results in increased synthesis of glutamate from 2-oxoglutarate and NH_3 (23). It has been suggested (5, 13) that glutamate functions as a counterion for K^+, which is taken up and accumulated upon an increase of the medium osmolarity. Also in *E. coli*, K^+ and its counterions (e.g., glutamic acid) can only give protection against a low level of osmotic stress (5, 12).

Externally supplied proline or betaine were accumulated to high concentrations in osmotically stressed *R. sphaeroides*. However, the addition of proline did not result and the addition of betaine did result in a significant increase in the growth rate under conditions of osmotic stress (i.e., the presence of 0.2 M NaCl). It has also been observed in other bacteria that betaine is a more efficient osmoprotectant than

proline is (22). These observations can possibly be explained by the greater ability of betaine to protect macromolecules against denaturation by high ionic strengths (8, 17).

Aerobic growth of *R. sphaeroides* under conditions of osmotic stress was stimulated by choline and betaine, while under anaerobic growth conditions in the light, only betaine was effective. Tracer experiments showed that choline serves as a precursor for the osmoprotectant betaine, which accumulated in osmotically stressed cells. The dipolar character of betaine, which allowed it to be accumulated in the cytoplasm without counterions, appears to be crucial for its osmoprotective capability. Although choline could be taken up under conditions of anaerobic growth in the light, no conversion to betaine occurred. This indicated that choline is converted to betaine in *R. sphaeroides* via an oxygen-dependent pathway. In *E. coli* choline was converted to betaine, with betaine aldehyde as an intermediate, via the osmotically regulated, oxygen-dependent choline-betaine pathway (11).

Only one constitutive choline transport system with a K_t value of 2.4 to 3.0 μM was present in *R. sphaeroides*, and no additional transport systems were induced by the presence of choline. The rate of choline uptake (V_{max} of 5.4 nmol of choline per min · mg of protein) was highest in cells grown in a low-osmolarity medium. A sudden increase in the medium osmolarity (upshock) inhibited choline transport, after which a gradual chloramphenicol-insensitive increase in the uptake rate of choline was observed. This indicated that the increase in transport activity is not caused by de novo protein synthesis; it is most likely due to restoration of cell turgor pressure by the accumulation of K^+.

R. sphaeroides possesses one constitutive betaine transport system which is activated at increased osmotic strengths. The synthesis of this transport system was not induced by osmotic stress since the immediate increase in betaine transport in low-osmolarity-grown cells after an upshock was also observed in the presence of chloramphenicol. A K_t of 18.2 μM and a V_{max} of 9.2 nmol/min · mg of protein were determined for betaine transport in cells grown in a high-osmolarity medium (0.3 M NaCl). This system was also able to transport L-proline, but with a lower affinity than that for betaine.

Betaine transport has been studied most extensively in *E. coli* and *Salmonella typhimurium* and has been found to be quite similar in the two organisms (3, 4). Two genetically distinct transport systems exist: a high-affinity system encoded by *proU* and a low-affinity system encoded by *proP* (3, 4). The inducible, high-affinity system (*proU*) is binding protein dependent, and both the synthesis and the activity of the transport system are increased at high osmolarities (3). Both betaine and proline are taken up by the constitutive *proP* system. This system functions most likely as an ion symporter, and activation of *proP* by a hyperosmotic shift has been demonstrated in whole cells and membrane vesicles of *E. coli* (16).

Activation of betaine transport at increased osmotic strengths was observed in *E. coli* (see above). A possible mechanism could involve deformation of the cell membrane because of changes in the turgor pressure. This, in turn, led to a conformational change in the transport protein and stimulation of betaine uptake. Different mechanisms by which betaine protects the cell against osmotic stress have been suggested. It may provide an osmotic balance across the membrane, and in addition, it can specifically protect enzymes against high salt concentrations (9, 13). The mechanisms by which betaine protects *R. sphaeroides* against osmotic stress are unclear.

ACKNOWLEDGMENTS

We thank W. Crielaard and A. J. M. Driessen for critical reading of the manuscript.

This work was supported by The Netherlands Foundation for Chemical Research, which is subsidized by The Netherlands Organization for the Advancement of Pure Scientific Research.

LITERATURE CITED

1. **Abee, T., K. J. Hellingwerf, and W. N. Konings.** 1988. The relation between electron transfer, proton motive force and lactose transport in membrane vesicles from aerobically grown *Rhodobacter sphaeroides*. Biochim. Biophys. Acta **932**:250–257.
2. **Baldwin, W. W., M. J. T. Sheu, P. W. Bankston, and C. L. Woldringh.** 1988. Changes in buoyant density and cell size of *Escherichia coli* in response to osmotic shocks. J. Bacteriol. **170**:452–455.
3. **Barron, A., J. U. Jung, and M. Villarejo.** 1987. Purification and characterization of a glycine betaine binding protein from *Escherichia coli*. J. Biol. Chem. **262**:11841–11846.
4. **Cairney, J., I. R. Booth, and C. F. Higgins.** 1985. *Salmonella typhimurium proP* gene encodes a transport system for the osmoprotectant betaine. J. Bacteriol. **164**:1218–1223.
5. **Dinnbier, U., E. Limpinsel, R. Schmid, and E. P. Bakker.** 1988. Transient accumulation of potassium glutamate and its replacement by trehalose during adaptation of growing cells of *Escherichia coli* K-12 to elevated sodium chloride concentrations. Arch. Microbiol. **150**:348–357.
6. **Epstein, W.** 1986. Osmoregulation by potassium transport in *Escherichia coli*. FEMS Microbiol. Rev. **39**:73–78.
7. **Giaever, H. M., O. B. Styrvold, I. Kaasen, and A. R. Strom.** 1988. Biochemical and genetic characterization of osmoregulatory trehalose synthesis in *Escherichia coli*. J. Bacteriol. **170**: 2841–2849.
8. **Hand, S. C., and G. N. Somero.** 1982. Urea and methylamine effects on rabbit muscle phosphofructokinase. J. Biol. Chem. **257**:734–741.
9. **Higgins, C. F., J. Cairney, D. A. Stirling, L. Sutherland, and I. K. Booth.** 1987. Osmotic regulation of gene expression: ionic strength as an intracellular signal? TIBS **12**:339–344.
10. **Higgins, C. F., L. Sutherland, J. Cairney, and I. R. Booth.** 1987. The osmotically regulated pro U locus of *Salmonella typhimurium* encodes a periplasmic betaine-binding protein. J. Gen. Microbiol. **133**:305–310.
11. **Landfald, B., and A. R. Strom.** 1986. Choline-glycine betaine pathway confers a high level of osmotic tolerance in *Escherichia coli*. J. Bacteriol. **165**:849–855.
12. **Larsen, P. I., L. K. Sydnes, B. Landfald, and A. R. Strom.** 1987. Osmoregulation in *Escherichia coli* by accumulation of organic osmolytes: betaines, glutamic acid, and trehalose. Arch. Microbiol. **147**:1–7.
13. **Le Rudulier, D., A. R. Strom, A. M. Dandekar, L. T. Smit, and R. C. Valentine.** 1984. Molecular biology of osmoregulation. Science **224**:1064–1068.
14. **Lowry, O. H., H. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
15. **May, G., E. Faatz, M. Villarejo, and E. Bremer.** 1986. Binding protein dependent transport of glycine betaine and its osmotic regulation in *Escherichia coli* K12. Mol. Gen. Genet. **205**: 225–233.
16. **Milner, J. L., S. Grothe, and J. M. Wood.** 1988. Proline porter 2 is activated by a hyperosmotic shift in both whole cells and membrane vesicles of *Escherichia coli* K-12. J. Biol. Chem. **263**:14900–14905.
17. **Pollard, A., and R. G. Wyn Jones.** 1979. Enzyme activities in concentrated solutions of glycine betaine and other solutes. Planta **144**:291–298.

18. **Poolman, B., E. J. Smid, H. Veldkamp, and W. N. Konings.** 1987. Bioenergetic consequences of lactose starvation for continuously cultured *Streptococcus cremoris*. J. Bacteriol. **169:** 1460–1468.
19. **Roth, W. G., S. E. Porter, M. P. Leckie, B. E. Porter, and D. N. Dietzler.** 1985. Restoration of cell volume and the reversal of carbohydrate transport and growth inhibition of osmotically upshocked *Escherichia coli*. Biochim. Biophys. Res. Commun. **126:**442–449.
20. **Speed, D., and M. Richardson.** 1968. Chromatographic methods for the isolation and identification of the products of choline oxidation. J. Chromatogr. **35:**497–505.
21. **Strom, A. R., P. Falkenberg, and B. Landfald.** 1986. Genetics of osmoregulation in *Escherichia coli*: uptake and biosynthesis of organic osmolytes. FEMS Microbiol. Rev. **39:**79–86.
22. **Sutherland, L., J. Cairney, M. J. Elmore, I. R. Booth, and C. F. Higgins.** 1986. Induction of the *proU* betaine transport gene is dependent on the accumulation of intracellular potassium. J. Bacteriol. **168:**805–814.
23. **Tempest, D. W., J. L. Meers, and C. M. Brown.** 1970. Influence of environment on the content and composition of microbial free amino acid pools. J. Gen. Microbiol. **64:**171–185.