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Different Mechanisms of Energy Coupling for Transport of Various Amino Acids in Cells of *Mycobacterium phlei**

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Whole cells of *Mycobacterium phlei* were shown to actively accumulate proline, leucine, lysine, tryptophan, histidine, glutamine, and glutamic acid to different steady state levels. The transport of proline, in contrast to that of other amino acids, was found to be insensitive to various respiratory inhibitors, *e.g.* cyanide, arsenate, azide, and sulfhydryl reagents. However, oxygen was an obligatory requirement for the uptake of proline, as well as for the other amino acids. The results indicate that the energy requirements for proline uptake are different from those of other amino acids. In contrast to the system from *Escherichia coli*, the mode of energy transduction for the uptake of proline, glutamine, and glutamic acid is different even though these amino acids are shock resistant in the *M. phlei* system.

The accumulation of various amino acids against a concentration gradient within a bacterial cell requires the expenditure of energy (1–4). Despite several recent studies in bacterial systems, the molecular mechanism by which energy is coupled to active transport remains an intriguing problem (3–6). Previous studies with membrane vesicles of *Mycobacterium phlei* showed that the uptake of proline, glutamine, and glutamic acid could be driven by oxygen uptake in the absence of energy derived from oxidative phosphorylation (1, 2, 7). Furthermore, no apparent correlation between the rate of substrate oxidation and the level of accumulation of these amino acids was observed (8, 9). The uptake of proline has been shown to differ from that of glutamine and glutamate, since in contrast to the latter amino acids, the uptake of proline requires Na^+ ions (7). In addition, the uptake of proline in membrane vesicles of *M. phlei* has also been shown to require specific phospholipids, whereas the uptake of glutamine and glutamic acid in the same system does not exhibit this requirement (10).

In *Escherichia coli* cells, Berger (11) has indicated that proline uptake is driven directly by an energy-rich membrane state which can be generated either by electron transport or ATP hydrolysis. Glutamine uptake, on the other hand, is apparently driven directly by phosphate bond energy generated by way of oxidative or substrate level phosphorylation. Based on the effects of osmotic shock, Berger and Heppel (12) recently showed that two categories of transport systems exist and concluded that the energy donors for the two classes are fundamentally different in *E. coli* cells.

The present communication describes some of the differences in the mode of energy transduction for the uptake of various amino acids in *M. phlei*. It has been shown that the uptake of proline was insensitive to respiratory inhibitors, arsenate and sulfhydryl reagents, while the uptake of leucine, histidine, lysine, tryptophan, glutamine, and glutamic acid was severely inhibited by these inhibitors. In contrast to *E. coli* (11, 12), the energy requirements for the uptake of proline in *M. phlei* cells was found to be essentially different from those of glutamine and glutamic acid, although the carrier(s) for proline, glutamine and glutamic acid are shock-resistant (4).

MATERIALS AND METHODS

Preparation of Cells—Mycobacterium phlei ATCC 354 was grown at 37° in 45 mM potassium phosphate, pH 7.0, 37 mM ammonium chloride, and supplemented with the following salts per liter of the media: 0.6 g of $MgSO_4 7H_2O$; 0.025 g of $ZnSO_4 7H_2O$; 0.1 g of $FeCl_3 6H_2O$; 0.02 g of $CaCl_2 2H_2O$; 0.002 g of $CoCl_2 6H_2O$; 0.001 g of $MnCl_2 4H_2O$; and 0.2% of Tween-80 was added to the growth media as a carbon source. The cells were grown to midlog phase (80 to 120 Klett units), quickly chilled, and centrifuged at 1500 × *g* for 10 min. The cells were washed three times in ice-cold 50 mM Hepes[1]-KOH, pH 7.4, containing 0.2% Tween-80 and were resuspended in the same buffer to a protein concentration of 0.2 to 0.3 mg/ml. Tween-80 was always kept in the suspending media in order to avoid clumping of the cells. Unless otherwise mentioned, 200 μg/ml of rifampicin was added to the suspension. After incubation for 30 min at 30°, these cells were used immediately for transport assay; although only slight loss in transport activity was observed during storage up to 4 to 6 hours at 4°.

Transport Assay with Intact Cells—Rifampicin-treated cells were incubated for 10 min at 30° and the uptake of amino acid was initiated by the addition of the desired concentration of ^{14}C-labeled amino acid. At indicated time intervals, 0.2-ml aliquots of cell suspension were removed with an Eppendorf pipette and immediately diluted in 5 ml of chilled phosphate buffer (0.05 M, pH 7.0). The suspension was rapidly

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[1] The abbreviations used are: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; *m*-CCP, *m*-chlorocarbonylcyanide phenylhydrazone; DTNB, 5-5'-dithiobis(2-nitrobenzoic acid); *p*CMBS, *p*-chloromercuribenzene sulfonic acid.

filtered on 0.45 μM Millipore filters. Dilution and filtration took less than 10 s for completion. Radioactivity retained by the filters was counted with a Nuclear Chicago liquid scintillation detector using a scintillation fluor with Beckman Bio-Solv (BBS-3).

Paper chromatography and thin layer chromatography of cell extracts, essentially as described by Piperno and Oxender (13), showed that the intracellular amino acids accumulated during transport assays were not chemically modified or incorporated into protein.

Measurement of Oxidation—The rate of oxidation by intact cells was measured polarographically at 30° with an oxygen monitor (14). No effect of oxidizable substrates was observed since the cells were already respiring (from an endogenous source) and attempts to deplete these endogenous substrates by aeration or by dinitrophenol (5 mM) treatment (12) were unsuccessful.

Assay of Transport under Anaerobic Conditions—To study transport under anaerobic conditions, Thunberg tubes were used. Oxygen was removed from the reaction system with a vacuum pump, and the sealed environment was saturated with argon gas. This was repeated three times to ensure a condition of anaerobiosis. The tubes were incubated in a shaking water bath at 30° for 10 min. The reaction was initiated by the addition of the indicated amount of ^{14}C-labeled amino acid from the side arm. At the time intervals indicated, the incubation contents were diluted and filtered as described above.

Irradiation of Cells—The cell suspension (200 μg of protein/ml) was irradiated for 40 min at 360 nm as described by Brodie *et al.* (15).

Measurement of ATP Levels—To measure ATP levels, 1.0 ml of the sample to be assayed was rapidly mixed with 0.1 ml of ice-cold 1.2 N perchloric acid and chilled for 30 min. After centrifugation, the decanted supernatant was neutralized and assayed for ATP levels using luciferin-luciferase assay (16).

Sulfhydryl Group Determination—The sulfhydryl groups were determined by the method of Ellman using DTNB (17).

Protein Determination—The cells were precipitated with 10% trichloroacetic acid and centrifuged at 2000 × *g* for 5 min. The pellet was resuspended in 1 N NaOH and heated at 80 to 90° for 5 min to ensure solubilization. Protein was estimated by the method of Lowry *et al.* (18).

RESULTS

Transport of Different Amino Acids in Whole Cells—Seven different amino acids, including acidic, basic, neutral, and aromatic, were chosen to study the mechanism of energy coupling for their uptake against a concentration gradient. Transport of proline, leucine, lysine, histidine, tryptophan, glutamine, and glutamic acid was followed. As seen in Fig. 1, these amino acids were accumulated to different levels in intact cells in the following order: lysine > proline > histidine > glutamic acid > glutamine > leucine > tryptophan. As mentioned in the experimental procedure, no exogenous substrate was added for the uptake of these amino acids since the cells were actively respiring; however, under the assay conditions, the total trichloroacetic acid-precipitable proteins failed to indicate that the amino acids tested were into protein to a significant level. In no case was the total incorporation of any of the amino acids into trichloroacetic acid-precipitable proteins more than 10% of the total accumulated amino acid.

Effects of Cyanide, Arsenate, and Azide on Uptake of Various Amino Acids—A 10 mM concentration of cyanide was able to block 95% of the total oxidation of intact cells (Table I). However, under such conditions, the uptake of proline remained unaffected, while the transport of other amino acids was reduced by 66 to 92% (Fig. 2*B* and Table II). Contrary to the inhibitory effect of arsenate on protein biosynthesis and phosphorylation, addition of arsenate did not significantly affect the capacity to accumulate proline. However, it affected the transport of leucine by 56% (Fig. 2) and of various other amino acids from 40 to 70% (Table II). These per cent values were calculated after subtracting trichloroacetic acid-precipitable incorporation of amino acid from total accumulation. The intracellular ATP levels decreased by 98% upon the addition of arsenate (Table I). The initial rate of proline uptake was the same in the presence and absence of arsenate (data not shown). A relatively long period of exposure (2 to 4 hours) of cells to arsenate prior to uptake measurement did not lower the steady state level of proline uptake.

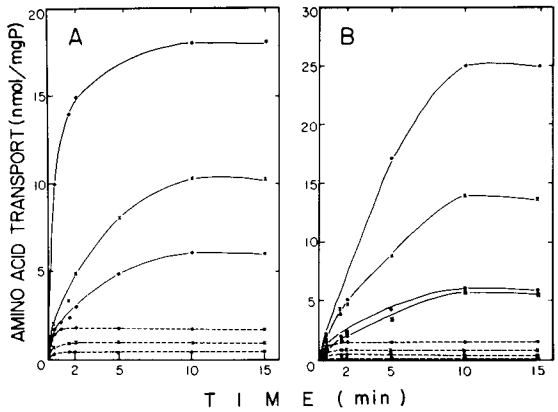

FIG. 1. Transport of proline, leucine, histidine, lysine, tryptophan, glutamine, and glutamic acid in *Mycobacterium phlei* cells. *M. phlei* cells were suspended in 50 mM Hepes-KOH buffer (pH 7.4) and incubated with rifampicin (200 μg/ml) for 30 min. Rifampicin-treated cells were used to assay the uptake of various amino acids. The uptake was initiated by the addition of labeled amino acids. The total input of labeled amino acids was 17 nmol/ml of assay system. For the determination of the incorporation of various amino acids into trichloroacetic acid-precipitable fractions, an aliquot of the sample was diluted to 25 times in chilled 10% trichloroacetic acid and filtered immediately on 0.45-μm Millipore filters. The *dashed lines* represent the incorporation of each amino acid into trichloroacetic acid-precipitable proteins. *A*, ●——●, proline; ×——×, glutamic acid; ○——○, glutamine. *B*, ●——●, lysine; ×——×, histidine; ○——○, leucine; ×——×, tryptophan.

TABLE I

Effect of various inhibitors on respiration and ATP levels in Mycobacterium phlei cells

M. phlei cells were suspended in 5 mM Hepes-KOH buffer (pH 7.4) at 200 to 300 μg of protein concentration and incubated with indicated amount of inhibitors for 10 min. The effect of these inhibitors on oxidation was monitored polarographically at 30°, and later, aliquot from the same reaction system was used to measure ATP levels. The ATP levels were measured using luciferin-luciferase (16)

Inhibitors	Concentration	% Inhibition of oxidation	ATP levels
	mM		*nmol/μg cell protein*
None		0	23.70
Arsenate	50	7.5	0.50
Cyanide	10	95.0	0.72
Azide	200	93.5	0.50

Uptake of proline was also insensitive to azide and *m*-chlorocarbonylcyanide phenylhydrazone. At 200 mM concentration, azide blocked oxidation and drastically reduced intracellular ATP levels (Table I). Under these conditions, the transport of proline remained unaffected as compared to the transport of leucine (Fig. 2, *A* and *B*) and other amino acids (Table II). The variable insensitivity to inhibitors for the uptake of these amino acids in *Mycobacterium phlei* cells was not due to an insufficient concentration of these inhibitors, as there was no further effect when the concentration of these inhibitors was increased.

Uptake of Proline and Leucine under Anaerobic

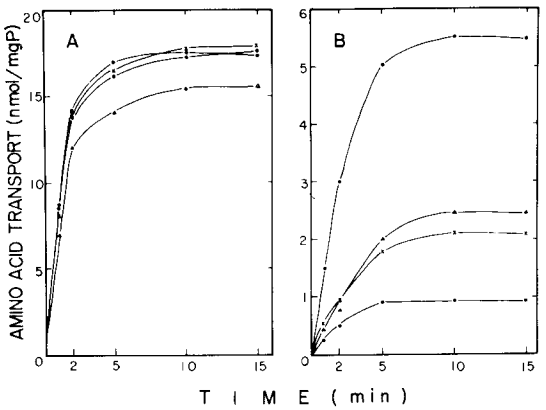

FIG. 2. Effect of various inhibitors on proline and leucine transport in *Mycobacterium phlei* cells. Rifampicin-treated cells in 50 mM Hepes-KOH buffer (pH 7.4) were incubated with either 50 mM sodium arsenate, 10 mM potassium cyanide, 200 mM sodium azide, or 100 μM *m*-CCP for 10 min, and then the uptake of proline and leucine was followed as described under "Experimental Procedure." *A*, proline uptake: ●——●, none; O——O, KCN; ×——×, azide; Δ——Δ, arsenate. *B*, leucine uptake: ●——●, none; O——O, KCN; ×——×, azide; Δ——Δ, arsenate.

TABLE II

Effect of various inhibitors on the uptake of different amino acids

The concentrations and conditions of inhibitors were the same as described in Table I. *m*-CCP was used at a final concentration of 100 μM. The measurement of transport was followed as described in experimental procedure.

Amino acid	Steady state levels of different amino acids				
	None	Azide	Arsenate	Cyanide	*m*-CCP
	nmol/mg protein				
Tryptophan	6.0	3.0	3.5	2.0	2.0
Histidine	13.0	4.5	4.0	1.0	1.5
Glutamine	6.0	2.5	2.0	1.0	2.0
Glutamic acid	11.0	8.0	7.0	1.0	2.0
Lysine	24.0	13.0	10.0	3.0	3.2

Conditions—Since the transport of proline was found to be insensitive to cyanide, arsenate, azide, and *m*-CCP, it was of interest to determine whether oxygen was an obligatory requirement for the uptake of proline. As can be seen from Fig. 3, the uptake of proline and leucine was completely inhibited under anaerobic conditions. The uptake of glutamine, glutamic acid, histidine, lysine, and tryptophan was also completely inhibited by anoxia (data not shown). Since oxygen was required for the uptake of proline, it is possible that the energy required for uptake of proline was derived from a bypass of the respiratory chain utilizing oxygen as terminal electron acceptor.

Effect of Irradiation on Proline and Leucine Transport—Irradiation of membrane vesicles of *M. phlei* at 360 nm has earlier been shown to inactivate the natural quinone MK-9 (II-H) and a light-sensitive factor of the respiratory chain (19). The irradiated membrane vesicles are unable to oxidize succinate and NAD^+-linked substrates (19). Whole cells of *M. phlei* were irradiated at 360 nm for 40 min and their oxidation was followed. Oxidation was found to be 90% inhibited in the irradiated cells. Transport of proline remained unaffected, while the transport of leucine was 90% reduced in these irradiated cells (Fig. 4). The transport of other amino acids was also inhibited 90 to 95% by irradiation (data not shown). It was

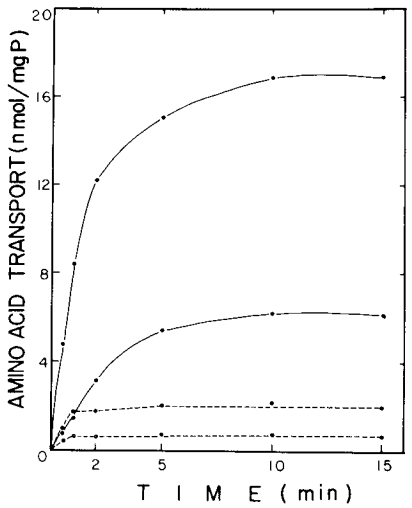

FIG. 3. Effect of anaerobiosis on proline and leucine transport. Anaerobiosis was done in Thunberg tubes as described under "Experimental Procedure." Other conditions for the assay of transport were similar to those described in Fig. 1. ●——●, proline; O——O, leucine; ●- - -●, proline (absence of O_2); O- - -O, leucine (absence of O_2).

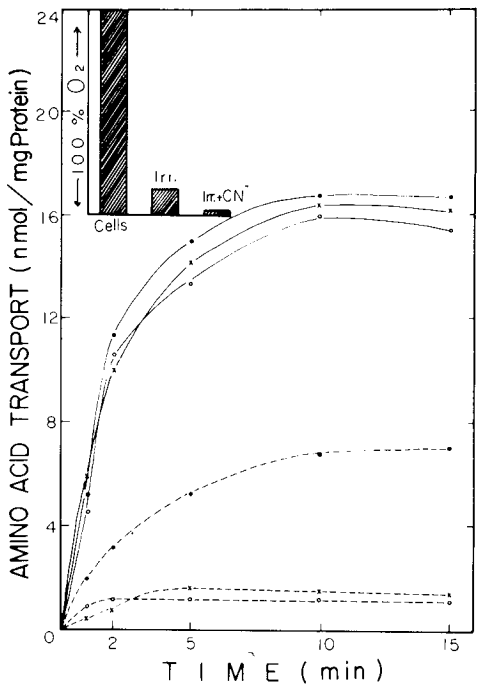

FIG. 4. Effect of irradiation on proline and leucine transport. Rifampicin-treated cells (200 μg of protein/ml) were irradiated at 360 nm of light as described elsewhere (20). Irradiated cells were checked polarographically for oxidation. The uptake of proline and leucine was assayed as described under "Experimental Procedure." Where indicated, 10 mM potassium cyanide was added to the cells and incubated for 10 min before assaying the uptake of proline and leucine. The *inset* in the figure shows per cent of oxidation before and after irradiation. The *solid lines* represent proline uptake, and the *dashed lines* represent leucine uptake in the presence or absence of inhibitors. ●——●, none; O——O, irradiated cells; ×——×, KCN + irradiated cells. ●- - -●, none; O- - -O, irradiated cells; ×- - -×, KCN + irradiated cells.

observed that the addition of cyanide to the irradiated cells blocked the remaining 10% oxidation, although the transport of proline remained unaffected (Fig. 4). Therefore, the possibility of a bypass which could support the uptake of proline using

oxygen as terminal electron acceptor was excluded by these irradiation experiments.

Sulfhydryl Group Contents under Aerobic and Anaerobic Conditions—Since anoxia was found to affect the uptake of proline, it is possible that anaerobiosis may affect the available sulfhydryl groups of the carrier(s) involved in proline transport. The total number of sulfhydryl groups under aerobic and anaerobic conditions was determined. It was observed (Table III) that the total number of DTNB-reactive sulfhydryl groups were decreased under anaerobic conditions. The decrement in sulfhydryl groups under anoxia could not be a result of oxidation of carrier(s) containing sulfhydryl moieties, but probably indicates a change in conformation of the carrier(s), resulting in the burial of —SH groups, thus making them inaccessible to DTNB reagent.

Effect of Sulfhydryl Inhibitors—The effect of sulfhydryl reagents, *e.g.* *p*CMBS and *N*-ethylmaleimide on the transport of proline and other amino acids was determined. It was possible to obtain a concentration of both *p*CMBS and *N*-ethylmaleimide at which there was no effect of oxidation, although the transport of various amino acids was affected. The presence of two distinct kinds of sulfhydryl groups has already been postulated from the studies on membrane vesicles obtained from *M. phlei* cells (8).

As seen from Table IV, the transport of proline remained unaltered by *N*-ethylmaleimide and *p*CMBS. Higher concentrations of sulfhydryl agents also did not affect the uptake of proline. However, the transport of leucine was 82% inhibited by *p*CMBS and 66% by *N*-ethylmaleimide. The inhibition in the transport of other amino acids by *p*CMBS and *N*-ethylmaleimide varied from 40 to 90% (Table IV). There does not appear to be a permeability problem for these inhibitors to accumulate in the *M. phlei* cells, since they were capable of blocking the oxidation and uptake of all of the amino acids tested except for proline. The sulfhydryl agents were also unable to elicit an efflux of proline when added at a steady state uptake level, but were effective in effluxing leucine and the other amino acids (Fig. 5).

Effects of Ionophores on Proline and Leucine Uptake—As compared to other amino acids, the transport of proline was observed to be insensitive to the respiratory inhibitors: cyanide, azide, irradiation of cells at 360 nm, and sulfhydryl agents. The possibility that an energized state of the membrane, or a threshold of membrane potential, or a combination of both, might be required in driving proline transport against a concentration gradient, was explored by using membrane permeable reagents such as dibenzyldiammonium, tetraphenylboron, or valinomycin. As seen in Table V, proline transport was 53 to 60% inhibited in the presence of the cationic membrane permeable substances, dibenzyldiammonium ions, tetraphenylarsonium ions, and valinomycin in the presence of K^+. The anionic permeable reagents, tetraphenylboron and tetraphenylphosphonium, had no effect on the proline uptake. The transport of leucine was inhibited by both cationic and anionic membrane-permeable reagents (Table V), as was the uptake of glutamine, glutamic acid, tryptophan, histidine, and lysine (data not shown).

TABLE III

Sulfhydryl content of Mycobacterium phlei cells under aerobic and anaerobic conditions

Cells denatured with trichloroacetic acid (2 mg of protein) were stirred with 2.5 ml water, 2 ml of 0.1 M phosphate buffer (pH 8.0), and 0.5 ml of a solution of 5-5'-dithiobis(2-nitrobenzoic acid) containing 39.6 mg of the reagent in 10 ml of 0.1 M phosphate buffer (pH 7.0). After 10 min, the samples were centrifuged and the intensity of the yellow color in the supernatant solution was measured spectrophotometrically at 412 nm. For direct determination, the treatment with trichloroacetic acid was omitted. For the determination of sulfhydryl groups under anaerobic conditions, Thunberg tubes were used as described under "Experimental Procedure." The values represent an average of four determinations.

Cells	Sulfhydryl content	
	Direct	Trichloroacetic acid treatment[a]
	nmol/mg protein	
Aerobic	18.8	42.0
Anaerobic	14.0	42.5

[a] Protein sedimented after trichloroacetic acid treatment.

DISCUSSION

The data presented show that in intact cells of *M. phlei* the transport of proline, in contrast to that of leucine, glutamine, glutamate, lysine, histidine, and tryptophan, is insensitive to respiratory inhibitors and arsenate. It has been shown earlier

TABLE IV

Effect of sulfhydryl group inhibitors on transport of various amino acids

The sulfhydryl agents were incubated with cells for 15 min; the uptake of various amino acids was initiated by the addition of ^{14}C-labeled amino acids and followed for 15 min as described under "Experimental Procedure." *p*CMBS and *N*-ethylmaleimide were dissolved in 0.05 M Hepes (pH 7.0).

Amino acid	None	*p*CMBS (79 μM)	*N*-ethylmaleimide (100 μM)
		nmol/mg protein	
Proline	18.0	17.5	18.1
Leucine	6.5	1.2	2.2
Tryptophan	6.0	3.8	3.8
Lysine	23.2	2.5	6.2
Glutamine	7.2	4.1	4.0
Glutamic acid	11.0	5.5	5.0
Histidine	13.5	2.2	6.0

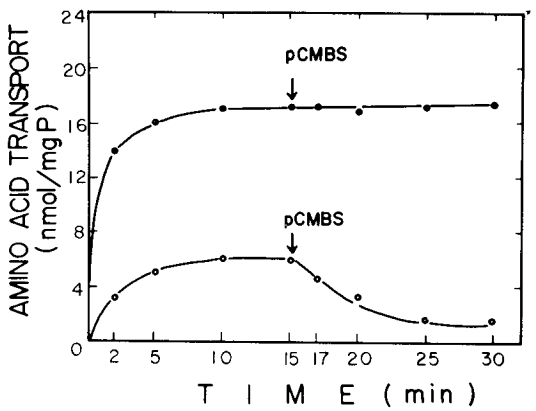

FIG. 5. Effect of *p*CMBS on steady state accumulation of proline and leucine. The transport of proline and leucine was initiated by the addition of labeled amino acid. After 15 min of incubation, 79 μM *p*CMBS was added to the reaction mixture and aliquots were taken every 2 min. The assay medium for the uptake was the same as described for Fig. 1. ●——●, proline; ○——○, leucine.

TABLE V

Effect of ionophores on proline and leucine transport

Mycobacterium phlei cells were suspended in 50 mM Hepes-KOH buffer (pH 7.4) at 200 to 300 μg of protein concentration and incubated with indicated amount of inhibitors for 10 min. The measurement of transport was followed as described under "Experimental Procedure."

Ionophores	Concentration	Proline	Leucine
		nmol/mg protein	
None		17	7
Dibenzyldiammonium	10 mM	7.8	2.8
Tetraphenylboron	100 μM	16.8	1.8
Valinomycin with K^+	20 μM	6.5	2.0
Tetraphenylarsonium	1 mM	8.0	2.0
Tetraphenylphosphonium	1 mM	17.0	2.6

that the uptake of proline in membrane vesicles and protoplast ghosts, prepared from whole cells of *M. phlei* grown in enriched media, was sensitive to respiratory inhibitors and insensitive to arsenate (1, 2). The uptake of proline in membrane vesicles prepared from Tween-grown cells was also sensitive to respiratory inhibitors. Therefore, differences in the sensitivity to respiratory inhibitors for proline uptake between membrane vesicles and intact cells were not due to a change in the growth media of *M. phlei* cells. A quantitative comparison between membrane vesicles and whole cells is somewhat difficult to reconcile, especially since the transport activity manifested by whole cells represents a composite of more than one system. Moreover, membrane vesicles prepared by sonic oscillation probably represent a perturbed system since the membrane vesicles, in addition to other possible differences, also differ from whole cells in size and vectorial orientation.

The uptake of different amino acids in intact cells of *M. phlei* was not affected by the addition of oxidizable substrates such as succinate, NADH, or ascorbate-TPD. These cells were actively respiring endogenous substrates and could not be depleted of endogenous respiration by dinitrophenol or starvation methods which have been used for other bacterial cells (12).

The sulfhydryl group inhibitors, *p*-chloromercuriphenyl sulfonic acid and *N*-ethylmaleimide, at concentrations which inhibit the respiration of cells, were found to be ineffective in blocking the uptake of proline, while the uptake of other amino acids (leucine, lysine, histidine, tryptophan, glutamine, and glutamic acid) was inhibited. The transport of all of these amino acids, except proline, was also inhibited by these sulfhydryl agents at a concentration at which there was no effect on respiration. This shows that in intact cells of *M. phlei*, there are different kinds of sulfhydryl groups involved in the respiration and transport of the following amino acids: lysine, leucine, histidine, tryptophan, glutamine, and glutamic acid. The presence of the two distinct kinds of sulfhydryl groups has already been postulated for *M. phlei* cells (8). Determination of sulfhydryl groups in intact cells showed that the number of DTNB-reactive sulfhydryl groups was reduced under anaerobic conditions as compared to the aerobic state. The decrease in the number of DTNB-reactive sulfhydryl groups indicates that the sulfhydryl groups are presumably becoming inaccessible to DTNB as a result of a change in conformation. The uptake of proline in intact cells appears to be independent of sulfhydryl groups, of carrier(s), or of those present in the vicinity of the carrier(s). Moreover, the carrier involved in the uptake of proline was different from that for other amino acids, since the uptake of proline was not affected by the presence of any other amino acid (data not shown).

Since the uptake of proline has an obligatory requirement for oxygen, it is possible that the energized state of the cell membrane is possibly maintained by oxygen. The energized state of the cell membrane could be maintained either by a proton gradient or an electrochemical gradient (membrane potential), or a combination of both. The relative contributions of the two components of proton motive force, a chemical gradient and membrane potential, can be calculated when no phenomenon other than H^+ movement is taking place. Movement of other ions across the membrane and the endogenous respiration has made it difficult to obtain definitive information concerning the mechanisms of active transport in whole cells of *M. phlei*. Since in *M. phlei* cells, the permeability of different ions is not known, the exact contribution of proton gradient and membrane potential to the electron motive force could not be ascertained.

In the present study, it was possible to measure the proton gradient by following the changes in the external pH of *M. phlei* cells under different assay conditions. The evidence that a proton gradient is not the driving force for proline uptake in *M. phlei* cells is supported by the following observations. Valinomycin, in the presence of K^+ ions and other membrane-permeable cations which have been shown to affect the membrane potential, inhibit proline uptake. However, uncouplers, such as *m*-CCP, potassium cyanide, and arsenate, which change the pH gradient (data not shown) do not affect proline uptake. The transport of other amino acids is, however, affected by both membrane-permeable cations and upcouplers, suggesting the involvement of membrane potential and proton gradient in the uptake of these amino acids.

Attempts were made to determine membrane potential, which presumably is the driving force for the uptake of proline, using fluorescent dye 3,3′-dihexyl-2,2′-oxacarbocyanine [$diOC_6(3)$] (20, 21) (data not shown). The true membrane potential could not be estimated using this fluorescent dye, since the endogenous respiration of cells could not be reduced. At present, different methods are under study to attempt to starve the cells which would then enable to determine the true membrane potential.

Berger and Heppel (12) have indicated that in *Escherichia coli* cells, the varying sensitivities of bacterial permeases to osmotic shock reflect underlying differences in energy coupling for different amino acids. However, in *M. phlei* cells, the osmotic shock treatment which results in the formation of protoplast ghosts (22) does not affect the transport of any amino acids,[2] suggesting that the carrier protein(s) for these amino acids are tightly bound as compared to *E. coli* cells (12). The carrier(s) involved in the uptake of proline, glutamine, and glutamic acid are tightly associated with the membrane since they are not lost even after sonication of either osmotically sensitive cells (protoplast ghosts) or whole cells (7). Although the carrier(s) for proline, glutamine, and glutamate proteins are resistant to shock (sonication), the mechanism for the uptake of these amino acids differs in whole cells as well as in membrane vesicles of *M. phlei*. Therefore, in attempting to delineate the mechanism of microbial active transport, species differences and differences in the mode of energy transduction for the uptake of different amino acids in the same system should be considered. In addition, the differences observed with different bacterial system in amino acid transport may be

[2] R. Prasad, V. K. Kalra, and A. F. Brodie, unpublished observation.

a reflection of their growth requirement for oxygen; for example, *E. coli* is a facultative bacterium while *M. phlei* is a strict aerobe.

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