The Assimilation of Amino-acids by Bacteria

9. THE PASSAGE OF LYSINE ACROSS THE CELL WALL OF *STREPTOCOCCUS FAECALIS*

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Gram-positive bacteria are able to concentrate certain amino-acids in the free state inside the cell prior to metabolism (Gale, 1947; Taylor, 1947). The passage of amino-acids across the cell wall into the cell may take place by a process of diffusion or by an active process requiring energy which can be obtained by exergonic metabolism (Gale, 1947). Studies of the passage of L-lysine across the cell wall of *Streptococcus faecalis* suggested that this amino-acid enters the cell by diffusion, since the entry will take place at 0°, is unaffected by metabolic inhibitors such as cyanides, iodoacetates and fluorides, takes place at a rate which is proportional to the external concentration, and has a temperature coefficient only slightly greater than that for the free diffusion of lysine. The concentration of lysine inside the cell is markedly greater than that outside the cell when the entry of lysine ceases. The curve relating the two concentrations suggests that an equilibrium is maintained between the internal and external environments. If this were the case, then it would be expected that lysine would leave a cell transferred from a high to a low external concentration. When, however, cells were grown in a high concentration of amino-acids and then incubated in a lysine-free salt medium, no lysine could be observed to leave the cell unless a source of energy such as glucose were added to the system. The present studies were undertaken in an attempt to determine whether an equilibrium does exist between the internal and external lysine concentrations and to investigate the conditions affecting that equilibrium.

METHODS

Streptococcus faecalis Dunn (National Collection of Type Cultures, no. 6783) was used throughout this study. The effect of phosphate ions on lysine migration was studied during growth and in washed suspensions. The basal medium for growth, supplemented by minimal amounts of amino-acids and phosphate and fortified by amino-acids and phosphate ions as the occasion demanded, had the following composition: Arginine, 0.50 g.; sodium acetate, 2.35 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.70 g.; KCl, 1.28 g.; ammonium acetate, 4.36 g.; glucose, 10.0 g.; ferrous ammonium sulphate 0.1 M, 0.15 ml.; marmite (P-free) 10%, 10.0 ml.; pyridoxin,

1.5 mg.; riboflavin, 1.5 mg.; calcium pantothenate, 3.0 mg.; and glass distilled water, 1000.0 ml.

The following were used as supplements when required: (1) phosphate, KH_2PO_4 , 4.3 g., and Na_2HPO_4 , 54.0 g./l. of basal medium; (2) amino-acids, phosphate-free casein hydrolysate 10.0 g./l. of basal medium. When this was added arginine was omitted from the basal medium. Phosphate was removed from the casein hydrolysate and from marmite as the magnesium ammonium salt. In each case the pH was adjusted to 7.4 and the culture incubated at 37° for 12–14 hr.

After harvesting, the cells were washed once with 100 ml. of water and made into a suspension in water of about 30–40 mg. dry wt. of cells/ml. For studies on lysine migration, 2.5 ml. of suspension were diluted in 25 ml. water, or 0.15 M-NaCl, Na_2SO_4 , Na_2HPO_4 , $\text{Na}_4\text{P}_2\text{O}_7$, with or without lysine 9.0 $\mu\text{mol./ml.}$

Lysine estimations were made manometrically using the specific decarboxylase (Gale, 1945), and the passage of lysine into the cells was followed by the technique described by Gale (1947). Quantities of lysine in the cells are expressed as $\mu\text{mol./100 mg. dry wt. of cells}$ and refer to free L-lysine.

RESULTS

Effect of phosphate on the accumulation of lysine by growing and resting cells

The ability of the cell to take up lysine from the medium and accumulate it in the free state within the cell during growth was studied. Organisms were grown in amino-acid-supplemented basal media containing minimal to high concentrations of phosphate ions. After an incubation of 12 hr. the cells were harvested, washed and their lysine content assayed. Table 1 shows that during growth the presence of

Table 1. *Effect of phosphate on the accumulation
of free lysine during growth*

(*Strep. faecalis* was grown on the basal medium containing 1% casein hydrolysate and supplemented with added phosphate as indicated.)

Exp.	Phosphate content of medium	Lysine content of cells ($\mu\text{mol./100 mg. drywt. of cells}$)
1	Traces	2.0
	0.15 M	5.5
2	Traces	3.4
	0.05 M	5.7
	0.15 M	9.1

phosphate favours the accumulation of lysine by the cell. This was not peculiar to lysine alone. In Exp. 1, 18.2 μ mol. of glutamic acid were accumulated per 100 mg. of cells in the presence of 0.15M-phosphate and only 4.5 μ mol. in the phosphate-deficient medium. In the case of glutamic acid this effect was to be expected, inasmuch as the migration of glutamic acid into the cell requires an energy-yielding reaction which necessarily requires adequate amounts of phosphate (Gale, 1947). With lysine, however, such is not the case.

The effect of phosphate on the accumulation of lysine by resting cells in washed suspension was then studied. Cells grown for 12 hr. in the basal medium with or without added phosphate were used. Their ability to accumulate lysine in washed suspensions was investigated at 4° and 37° in the presence or absence of phosphate. Table 2 shows that the accumulation of lysine was in each instance better in the absence of added phosphate, whether organisms were grown in phosphate-poor or phosphate-rich medium. Cells grown in phosphate-deficient medium accumulated appreciably more lysine than those grown in the presence of large amounts of phosphate.

The possibility remains that the organism might have accumulated lysine equally well in the presence of phosphate and that the lower levels observed might have been due to increased internal metabolism of the amino-acid under the influence of inorganic phosphate. The fact that the same phenomenon was observed when migration of lysine took place at 4° makes this unlikely. However, this possi-

bility was checked by an experiment, similar to those previously described for glutamic acid by Gale & Mitchell (1947), in which the lysine was assayed in the cell and in the medium before and after incubation. If any metabolism occurred during migration this would show as a loss of total lysine after incubation. It is clear from Table 3 that all the lysine lost from the medium was taken up by the cells and no metabolism could be detected.

The effect of phosphate on the migration of lysine from the internal to the external environment

From the foregoing it is obvious that the inward migration of lysine into the cell is influenced by inorganic phosphate in the external medium.

The diffusion of lysine into the medium from within the cells was next studied, and again the only ion that showed any influence on this outward migration was inorganic phosphate. Cells were grown in the deficient basal medium, and then incubated in lysine (9 μ mol./ml.) until a high internal concentration was attained.

Such lysine-filled cells were then allowed to equilibrate in 100 ml. of various salt media at pH 7.2. Table 4a shows that at 37° lysine migrates out of the cell equally well in either 0.15M-NaCl or 0.15M-Na₂HPO₄.

In similar experiments performed at 4° for 20 hr. instead of at 37° migration took place when the lysine-filled cells were incubated in saline solution, but not in the phosphate medium (Table 4b). Moreover, the amount of lysine lost from the cells was

Table 2. *Effect of phosphate on the diffusion of lysine into the resting cell*

(*Strep. faecalis* was grown for 12 hr. at 37° in the basal medium with and without added phosphate. Washed cells were suspended in lysine solutions (4.5 μ mol./ml.) with and without phosphate, and incubated for 2 hr. at 37° or 20 hr. at 4°.)

Exp.	Growth medium	Additions (each 0.15M) to lysine solution for incubation	Temp.	Lysine uptake (μ mol./100 mg. dry wt. of cells)
1	Basal	NaCl	37°	5.3
	Basal	Na ₂ HPO ₄	37°	3.8
	Basal + 0.15M-Na ₂ HPO ₄	NaCl	37°	3.8
	Basal + 0.15M-Na ₂ HPO ₄	Na ₂ HPO ₄	37°	3.0
2	Basal + 0.15M-Na ₂ HPO ₄	NaCl	4°	3.9
		Na ₂ HPO ₄	4°	2.8
		NaCl	37°	5.5
		Na ₂ HPO ₄	37°	2.0

Table 3. *Effect of phosphate on lysine accumulation by Streptococcus faecalis grown in an amino-acid- and phosphate-deficient medium*

(*Strep. faecalis* was grown in the deficient medium for 14 hr. at 37°. The washed cells were incubated in lysine solution for 2 hr. at 37° at pH 7.2. The lysine taken up by the cells and that left in the medium was determined.)

Medium (4 ml.)	Dry wt. of cells (mg.)	Lysine (μ mol.)			
		In medium before incubation (a)	In medium after incubation (b)	Taken up by cells (c)	Metabolized a - (b + c)
Lysine + 0.15M-NaCl	143	36.4	23.7	12.3	+ 0.4
Lysine + 0.15M-Na ₂ HPO ₄	143	36.4	28.8	8.2	- 0.6

Table 4. *Effect of ions on outward migration of lysine*

(*Strep. faecalis* was grown in the deficient medium for 14 hr. at 37°, washed and incubated in lysine 9 μ mol./ml. for 2 hr. at 37°. It was again washed and allowed to equilibrate in 100 mol. of various salt media as indicated. 2 hr. at 37° or 20 hr. at 4° was sufficient for attaining equilibrium between internal and external environments. All lysine values expressed as μ mol./100 mg. dry wt. of cells.)

Lysine content of cells after incubation in:

Conditions of incubation	Initial lysine content	NaCl (M)			Na ₂ HPO ₄ (M)			0.15 M-NaCl + 0.05 M-Na ₂ HPO ₄	0.15 M-Na ₂ SO ₄	0.15 M-Na ₄ P ₂ O ₇
		0.15	0.05	0.005	0.15	0.05	0.005			
(a) 2 hr. at 37°	10.5	7.0	—	—	7.4	—	—	—	—	—
	10.1	—	—	—	5.5	—	—	—	—	—
(b) 20 hr. at 4°	7.8	1.6	4.5	—	8.0	7.8	—	7.8	—	—
	6.9	3.1	—	7.1	7.2	—	7.2	—	—	—
(c) 20 hr. at 4°	12.7	8.0	—	—	—	—	—	—	—	7.8
	10.1	5.9	—	—	—	—	—	—	—	5.6
	13.7	6.1	—	—	13.8	—	—	—	6.5	—

recovered in the saline medium (Table 5). No lysine was detected in the phosphate medium and no loss of lysine observed in the cells. The effect of the phosphate ion in preventing the leakage of lysine at 4° was still exerted in the presence of NaCl (Table 4b).

Table 5. *Outward migration of lysine from cells suspended in saline*

(*Strep. faecalis* was grown in the deficient medium for 14 hr. at 37°. Washed cells were incubated with 9.0 μ mol./ml. lysine for 2 hr. at 37°, washed again and allowed to equilibrate in 100 ml. NaCl or Na₂HPO₄ at pH 7.2 for 20 hr. at 4°.)

Initial lysine content (μ mol./100 mg. dry wt. of cells)	Lysine (μ mol.)			
	After incubation in NaCl (0.15 M)		After incubation in Na ₂ HPO ₄ (0.15 M)	
	Cells	Medium	Cells	Medium
10.0	4.5	5.3	10.5	0.0
10.3	7.6	2.7	10.3	0.0
7.8	3.1	4.6	7.6	0.0

Other salts, sodium sulphate and sodium pyrophosphate, were tested in a similar manner. It was found that their effect on lysine migration to the

outside medium was like that of NaCl (Table 4c). It was thought possible that these ions might have caused a permanent alteration in the permeability of the cell wall toward lysine. Cells filled with lysine were therefore subjected to the action of various ions, and their ability to reaccumulate or lose lysine was then studied under various conditions. The results of such an experiment are shown in Table 6. There is no evidence that any damage was suffered by the cell while in contact with chloride, sulphate, pyrophosphate or phosphate since the cell, after treatment, continued to behave normally toward lysine migration.

THE EFFECT OF OTHER AMINO-ACIDS ON THE PASSAGE OF LYSINE ACROSS THE CELL WALL

The finding that at 37° lysine migrated equally well in phosphate as in saline is in contrast to the earlier finding (Gale, 1947) which has since been confirmed, that lysine failed to migrate at 37° to the outside medium containing phosphate, unless a source of energy was supplied.

Beside the difference in growth media used, one further difference between the two sets of observations is the re-

Table 6. *Reversible effect of ions on lysine accumulation*

(*Strep. faecalis* was grown in the deficient medium for 14 hr. at 37° and allowed to equilibrate with lysine 9.0 μ mol./ml. at 37° for 2 hr. The lysine-filled cells were then put through two successive incubations as indicated. Samples were taken for lysine assay. Incubations at 4 and 37° lasted 20 and 2 hr. respectively.)

Initial lysine content (μ mol./100 mg.)	First incubation			Second incubation		
	Medium (0.15 M)	Lysine content (μ mol./100 mg.)	Temp.	Medium	Lysine content (μ mol./100 mg.)	Temp.
10.3	NaCl	7.6	4°	Lysine (9.0 μ mol./ml.)	11.0	37°
10.1	Na ₄ P ₂ O ₇	7.9	4°	Lysine (9.0 μ mol./ml.)	13.5	37°
10.5	Na ₂ HPO ₄	10.5	4°	NaCl (0.15 M)	8.1	4°
10.7	Na ₂ SO ₄	8.0	4°	Lysine (9.0 μ mol./ml.)	11.6	37°

lative concentration of the free amino-acids within the cells. In the present instance, with growth on an amino-acid deficient-medium and preincubation with lysine, lysine was the only amino-acid present in high concentration, and outward migration took place freely in phosphate medium. In the earlier experiments (Gale, 1947) growth in an amino-acid-rich medium resulted in a high internal concentration of other free amino-acids, and migration did not take place in a phosphate medium. It seemed possible, therefore, that the presence of other amino-acids in the same environment with lysine might affect its migration across the cell membrane.

The effect was studied using cells grown for 12 hr. at 37° in a medium deficient only in amino-acids (medium B of Gale, 1947). The deficient cells were then allowed to take up lysine by incubation at 37° for 3 hr. in lysine solutions containing various combinations of amino-acids but all having 9 μ mol. of lysine per ml.

Table 7 shows that the basic or acidic amino-acids retarded lysine entry. The effect exerted by the

acidic mixture was more pronounced in each case. Combination of acidic and basic mixtures neither neutralized nor enhanced that effect. It seems probable that any effect shown by casein hydrolysate is due to the presence of the acidic and basic amino-acids, since the neutral ones had no detectable effect. It is necessary at this point to stress the fact that under these conditions the lysine entering the cell is not metabolized. Recovery experiments, using cells with 110 mg. dry weight, showed that, when lysine accumulation occurred upon incubation in casein hydrolysate, 7.0 μ mol. were added to the cell content and 6.6 μ mol. were lost from the medium. Upon incubation in a solution of lysine with glutamic and aspartic acids the internal lysine content rose by 9.8 μ mol., and 9.9 μ mol. were lost from the medium.

The effect on outward migration of lysine from the cell into phosphate or saline medium was investigated, using similarly grown deficient cells after they had taken up lysine during 3 hr. incubation at 37° with

Table 7. *The effect of other amino-acids on the accumulation of lysine*

(Streptococci were grown 12 hr. at 37° in basal medium and incubated for 2 hr. at 37° in the various suspension media at pH 7.2. The increase in lysine content was then measured as usual. The relative concentrations of the various amino-acids were approximately the same as in casein hydrolysate. Basic amino-acids (μ mol./ml.): lysine (9.0), arginine (4.0), histidine (4.0); acidic amino-acids (μ mol./ml.): glutamic (24.0), aspartic (9.0); neutral amino-acids (μ mol./ml.): alanine (3.5), valine (11.0), phenylalanine (4.0), proline (11.0), tryptophan (2.0), serine (1.5) glycine (2.0).)

Composition of the suspension medium (all containing 9 μ mol. of lysine/ml.)	Increase in free lysine content (μ mol./100 mg. dry wt. of cells)			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Lysine	18.3	18.5	15.6	16.7
Casein hydrolysate 2.5%	10.6	6.0	—	—
Lysine + basic amino-acids	12.1	7.0	9.9	—
Lysine + acidic amino-acids	6.5	4.5	7.6	—
Lysine + neutral amino-acids	—	—	14.8	15.0
Lysine + neutral + acidic amino-acids	—	—	—	5.2
Lysine + basic + acidic amino-acids	—	—	—	5.1

Table 8. *The effect of other amino-acids on the outward migration of lysine*

(*Strep. faecalis* was grown for 8 hr. in casein digest (1 and 2), in the basal medium (3 and 4), and medium B (5–9) for 12 hr. and allowed to take up lysine from various solutions containing 9 μ mol./ml. at pH 7.2, temp. 37°, duration 2 hr. It was subsequently incubated in phosphate and saline under the same conditions of temp., pH and time of incubation. Neutral, basic and acidic amino-acids as in Table 7.)

Exp.	Initial lysine content*	First incubation		Second incubation			
		Medium	Lysine content*	Na ₂ HPO ₄ (M)	Lysine content*	NaCl (M)	Lysine content*
1	7.0	—	—	0.15	7.2	0.15	5.0
2	9.2	—	—	0.075	9.1	0.15	5.3
3	1.5	Casein hydrolysate 2.5%	8.7	0.075	8.8	0.15	1.5
4	1.1	Casein hydrolysate 2.5%	7.2	0.15	7.1	0.15	4.5
5	1.4	Casein hydrolysate 2.5%	9.6	0.15	9.7	0.15	3.2
6a	1.7	Casein hydrolysate 2.5%	9.4	0.075	9.2	—	—
6b	1.7	As (3) + 1% glucose	5.0	0.15	5.0	0.15	2.1
7	1.3	Lysine	12.4	0.15	6.2	0.15	6.0
8	1.5	Lysine	13.0	0.075	4.2	0.15	4.4
9a	4.3	Lysine + acidic amino-acids	12.9	0.075	12.8	0.15	5.1
9b	4.3	Lysine + basic amino-acids	11.9	0.075	11.9	0.15	5.3
9c	4.3	Lysine + neutral amino-acids	15.8	0.075	7.1	0.15	5.0

* Lysine content in μ mol./100 mg. dry wt. of cells.

the various amino-acid combinations as described above. Equilibration took place at 37° for 2 hr. Similarly, streptococci grown on a casein-digest medium (Gale, 1947), already possessing high levels of free amino-acids, were used without prior incubation with lysine solution.

Table 8 shows that, in all cases where saline was used, free migration of lysine took place. However, with phosphate in the external medium, lysine did not migrate when it had been taken up, either during growth in an amino-acid rich medium or during incubation in the presence of the acidic or basic amino-acids or casein hydrolysate with or without glucose; but migration took place to the same extent as in saline when lysine had been taken up from lysine solution alone or in the presence of the neutral amino-acids. Again it appears that both the basic and acidic amino-acids exerted a definite effect on the diffusion of lysine out of the cell as well as into the cell.

DISCUSSION

We have shown above that the phosphate ion has a definite effect on the migration of lysine across the cell wall of *Strep. faecalis*. When the exchange across the cell barrier is considered in its simplest form, occurring at 4° with lysine as the only amino-acid and phosphate as the only anion, some aspects of this exchange are explicable along the lines of Donnan equilibria. Quantitative studies on the passage of ions showed that there is no leakage of phosphate out of the cell upon suspending the organism in water or saline. Further, there is no increase in the phosphate content (free or esterified) of the cells when these are suspended in 0.15M-phosphate with or without lysine. When lysine is taken up from an aqueous solution one can readily see that, on the internal side of the membrane, the presence of non-diffusible phosphate anion plus the negatively charged non-diffusible protein of the cell tends to favour the migration of the diffusible cation lysine into the cell, and to build up a final concentration therein at a level higher than that present in the outside medium. Further, the addition of non-diffusible inorganic phosphate ion to the external medium will tend to reduce the internal lysine concentration at equilibrium.

However, when the system is reversed and lysine-filled cells are suspended in a medium containing phosphate at 4° no detectable migration takes place even after 20 hr. Migration invariably occurs when saline is the outside medium, but not when phosphate ions are present in saline. The process is made still more complicated by effects shown by the acidic and

basic amino-acids. There is evidence that the acidic amino-acids do not diffuse across the cell wall (Gale, 1947) and would therefore act as external non-diffusible ions, but the basic amino-acids also have the effect of lowering the internal lysine concentration. There is as yet no definite evidence concerning the permeability of the cell wall towards basic amino-acids other than lysine.

SUMMARY

1. The passage of lysine across the cell membrane of *Streptococcus faecalis* has been investigated. Two factors appear to influence lysine migration: the phosphate ion and certain amino-acids.

2. Phosphate favours lysine accumulation by the streptococci during growth in a medium of high amino-acid content.

3. In washed suspensions of cells, lysine accumulation is less if the external medium contains phosphate.

4. The passage of lysine from the internal to the external environment of the cell occurs readily at 4° in the presence of chloride, sulphate or pyrophosphate ions, but no outward migration takes place in the presence of phosphate.

5. Outward migration takes place at 37° whether phosphate is present or absent.

6. The outward migration is influenced by the presence of other amino-acids during the previous uptake of lysine. If cells deficient in free amino-acids are incubated in lysine alone, then the lysine taken up can subsequently leave the cell if this is incubated in lysine-free phosphate at 37°. If the uptake of lysine takes place from a medium containing casein hydrolysate then the lysine will not leave the cell on subsequent incubation in phosphate although normal migration occurs in saline.

7. The retaining effect of casein hydrolysate is also obtained if the initial incubation takes place in a medium containing, in addition to lysine, a mixture of either arginine and histidine or glutamate and aspartate.

8. Uptake of lysine by deficient cells is also lessened by the presence of these amino-acid mixtures.

9. A mixture of neutral amino-acids has no effect on either the uptake of lysine or its subsequent migration out of the cell.

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