Transport and Excretion of L-Lysine in *Corynebacterium glutamicum*

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L-Lysine transport in *Corynebacterium glutamicum* was investigated. The bacterium was shown to possess a highly specific, energy-dependent system of active lysine transport. The system transferred lysine into the cells and exchanged intra- and extracellular lysine. Mutations in the transport system did not lead to overproduction of the amino acid. Resting cells of the parent strain, or of its lysine-producer derivatives with a defective transport system, failed to excrete lysine into the medium. An efflux of intracellular lysine could be induced by a hyperosmotic shock, and by different membrane-active substances. It has been suggested that *C. glutamicum* cells are equipped with channels (pores) for excreting lysine from the cytoplasm. These channels appeared to open in response to an increase in the intracellular lysine concentration. The channel permeability also depends on the membrane structure.

INTRODUCTION

Various bacteria used as industrial producers of a number of low molecular mass compounds, including amino acids, excrete these compounds into the cultivation medium. Obviously, the transfer of a water-soluble substance across the bacterial cytoplasmic membrane into the environment requires a special mechanism. Yet our knowledge on the appropriate mechanisms is rather limited.

Three excretion mechanisms are possible. One is associated with a change of membrane permeability for individual substances: for glutamate in glutamate-producing strains of *Brevibacterium lactofermentum* and *Corynebacterium glutamicum* (Demain & Birnbaum, 1968; Furuya *et al.*, 1969), and for 5'-inosinic acid in *Brevibacterium ammoniagenes* (Furuya *et al.*, 1969; Teshiba & Furuya, 1983). The permeability towards these substances depends on the presence of biotin and manganese ions, respectively.

The second mechanism is associated with the functioning of special transport proteins. A transport protein is supposed to be responsible for both the entry and exit of the substrate. If the affinity of such a carrier protein for the external substrate is reduced by mutation, the uptake of the substrate will stop but the efflux will continue, resulting in accumulation of the metabolite in the medium. This mechanism is thought to be responsible for the excretion of lysine by S-2-aminoethyl-L-cysteine (AEC)-resistant mutants of *Escherichia coli* (Halsall, 1975).

Finally, the third mechanism can, theoretically, be attributed to the functioning of specialized excretion systems. Thus, energy-dependent excretion of cAMP (Goldenbaum & Hall, 1979), calcium ions (Rosen & McClees, 1974), arsenate and some antibiotics (Foster, 1983) have been described in *E. coli*. A specific riboflavin excretase has been found in the yeast *Pichia guilliermondii* (Sibirny *et al.*, 1978).

C. glutamicum has long been used as an industrial producer of L-lysine (Kinoshita *et al.*, 1958;

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; PCP, pentachlorophenol; TPP⁺, tetraphenylphosphonium; TPMP⁺, triphenylmethylphosphonium; AEC, S-2-aminoethyl-L-cysteine; LHX, lysine hydroxamate; $\Delta\psi$, transmembrane electric potential.

Ozaki & Shiio, 1983). However, nothing has to our knowledge been published on transport and secretion of lysine by this micro-organism. An attempt is made in the present study to elucidate the mechanism(s) underlying lysine transport through the bacterial membrane.

METHODS

A wild-type *C. glutamicum* strain ATCC 13032 and mutants derived from it were used. Some details of the strains are given in Table 1. Spontaneous AEC-resistant mutants (S11 and S21) were selected from wild-type bacteria on minimal plates supplemented with glucose, 1 mg AEC ml⁻¹ and threonine. The rest of the mutants were selected after mutagenization with nitrosoguanidine. The mutants 34, 410 and 1-2-89, which are lysine producers, have an aspartokinase (the key enzyme of lysine biosynthesis) that is insensitive to feedback inhibition by a mixture of lysine and threonine (Kara-Murza *et al.*, 1978; Leonova *et al.*, 1981).

The cells were routinely grown at 30 °C to the exponential phase (4–5 h) in Nutrient broth (Difco) containing 5% glucose with aeration provided by a shaker. Then they were collected on a filter (HAWP, Millipore, 0.45 µm pore size, 48 mm diameter), washed with Tris/HCl (0.01 M, pH 7.3) and suspended in the same buffer. The optical density of the suspension (0.4–0.6) was measured with a KFO photocolormeter (530 nm maximum transmittance filter) in 5 mm lightpath cuvettes.

The rate of lysine transport was measured after adding radioactive lysine [330 mCi mmol⁻¹ (12.2 GBq mmol⁻¹) Amersham] to a bacterial suspension held at 30 °C. An automatic pipette was used to take 0.2–0.4 ml samples at certain time intervals; the cells were collected on fibreglass filters (Whatman GF/F) mounted on a Millipore multi-filter holder and washed with 10 ml Tris/HCl buffer. The filters were dried, placed in scintillation vials and their radioactivity was counted with a Delta 300 scintillation counter. Since [¹⁴C]lysine accumulation in cells was a linear function of incubation time up to 2 min (see Fig. 1), the value at the intersection of this line with the ordinate was taken as the amount of label adsorbed non-specifically by the filter. The appropriate correction was made in calculating the amount of lysine uptake.

To measure the intracellular lysine pool, the cells from 0.5 l exponential culture were collected by centrifugation and washed twice with Tris/HCl, pH 7.3. The pellet was kept in an ice bath with 1 ml 10% TCA for 30 min. The cells were then sedimented by centrifugation, and TCA was removed with ether. The lysine content of the extract was determined with a Durrum D500 amino acid analyser. According to Clement *et al.* (1984) the intracellular water volume is considered to be 1.76 µl (mg dry wt)⁻¹.

The membrane potential ($\Delta\psi$) was determined by using the lipid-soluble cation triphenylmethylphosphonium (TPMP⁺). The intracellular accumulation of [¹⁴C]TPMP⁺ [100 µCi ml⁻¹ (3.7 MBq ml⁻¹)] was studied by the same method as the transport of [¹⁴C]lysine. [¹⁴C]TPMP⁺ accumulation attained equilibrium 15 min after adding the cation to the cells. The value of $\Delta\psi$ was calculated from the Nernst equation using the equilibrium intracellular concentration of TPMP⁺, its outside concentration being 5.5 µM. The non-specific binding of [¹⁴C]TPMP⁺ was determined by using cells treated with 1% toluene. The average value of $\Delta\psi$ was 180 mV. Ethanol (0.5%) was used to dissolve some inhibitors. It did not affect the accumulation of [¹⁴C]lysine or [¹⁴C]TPMP⁺.

To study the efflux of lysine, a bacterial suspension was incubated in Tris/HCl (0.01 M, pH 7.3) in the presence of [¹⁴C]lysine (1 µM) for 40 min; the suspension was then quickly diluted 100-fold with the same buffer prewarmed to 30 °C. Samples (5 ml) were passed through fibreglass filters to collect and wash the cells.

Cells with no lysine pool were prepared by incubation with gramicidin S (10⁻⁵ M) for 10 min. The cells were collected on filters (HAWP, Millipore, 0.45 µm pore size) and washed with Tris/HCl (0.01 M, pH 7.3).

Cell proteins were labelled by the following procedure. Cells were grown in a glucose/salt medium for 24 h in the presence of 10 µM [¹⁴C]lysine. Then they were collected by centrifugation and washed with Tris/HCl (0.01 M, pH 7.3). Proteins were extracted by treating the cells with 5% TCA at 90 °C for 10 min. The TCA-insoluble pellets were collected on fibreglass filters and washed first with 5% TCA containing unlabelled lysine (10⁻² M) and then with ethanol. Radioactivity of probes was measured.

Compounds used in the study were obtained from the following sources: carbonyl cyanide *m*-chlorophenylhydrazine (CCCP), valinomycin, sodium azide, Triton X-100 and *N*-ethylmaleimide were from Serva; *N,N'*-dicyclohexylcarbodiimide (DCCD) and nigericin were from Calbiochem; pentachlorophenol (PCP) and rotenone were from BDH; monencin was from Lilly, and lysine analogues and nigericin were from Sigma.

Gramicidin S was kindly provided by Professor G. F. Gauze (Institute for Search of New Antibiotics, Moscow).

RESULTS

Lysine transport into the bacteria

In studying the transport of a substance, one usually tries to prevent its subsequent metabolism after it has entered the cytoplasm. This is done by using a non-metabolizable

Table 1. *Strains used in this study*

Strain	Parent strain	Phenotype	Sensitivity of aspartokinase to lysine + threonine*	Lysine production (g l ⁻¹)†	Intracellular lysine pool (mM)	Initial rate of lysine transport‡		Origin or reference
						pH 7.3	pH 9.5	
ATCC 13032	-	Wild-type	+	0.2	5	66	400	Collection of the Institute
S11	13032	AEC ^R	ND	0.3	4	2	2	This study
S21	13032	AEC ^R	ND	0.2	ND	3	3	This study
34	13032	AEC ^R	-	17	ND	14	11	Leonova <i>et al.</i> , (1981)
410	13032	AEC ^R	-	14	1	2.5	5	Kara-Murza <i>et al.</i> , (1978)
1-2-89	13032	LHX ^R	-	7	ND	5	22	This study
95	13032	Homoserine-dependent	+	30	5	60	300	Zaitseva & Chernysheva (1975)
10	95	Homoserine-dependent AEC ^R LHX ^R	-	35	60	20	20	This study

ND, Not determined.

* Aspartokinase is considered sensitive (+) to inhibition by a mixture of lysine and threonine (10 mM each) if its activity is inhibited by more than 80% and resistant (-) if the inhibition is less than 20%.

† Measured in the culture fluid after growing cells for 72 h in a mineral medium with glucose.

‡ Measured with 1 μ M [¹⁴C]lysine in the medium.

Fig. 1

Fig. 2

Fig. 1. Incorporation of $[^{14}\text{C}]$ lysine into *C. glutamicum* 13032 cells (●), into a TCA-insoluble fraction (■) and into cells treated with gramicidin S (time of addition shown by arrow) (▲). $[^{14}\text{C}]$ lysine concentration in the medium was $0.5\ \mu\text{M}$.

Fig. 2. Initial rate of $[^{14}\text{C}]$ lysine transport (●) into *C. glutamicum* 13032 cells, and their content of lysine zwitterion as calculated from the Henderson-Hasselbalch equation (○), as functions of pH. $[^{14}\text{C}]$ lysine concentration in the medium was $0.5\ \mu\text{M}$.

derivative of the substance or by introducing a mutation into the genes responsible for utilization. We, however, did not face such a problem. We found that $[^{14}\text{C}]$ lysine which entered *C. glutamicum* ATCC 13032 cells incubated in Tris/HCl was not subject to any subsequent degradation: 95% of the label extracted with TCA from *C. glutamicum* cells after 30 min incubation was found in the lysine spot after paper chromatography. At the same time label incorporation into acid-insoluble material was quite low under our conditions (Fig. 1), and the cells lost almost all their accumulated lysine when exposed to gramicidin S (Fig. 1). This implied that the substance was stored in an osmotically active state in the cell cytoplasm. The treatment of bacteria with cold TCA (see Methods) also indicated that the lysine pool was between 4 and 5 mM (Table 1). Nevertheless, the cells were able to accumulate $[^{14}\text{C}]$ lysine against a pre-existing gradient (Fig. 1).

The initial rate of uptake of label increased in proportion to the outside concentration which was varied from 0.1 – $5\ \mu\text{M}$. At higher concentrations the linear portion of the accumulation *vs* time curve extended over less than 5 s; therefore we were unable to determine the initial rate accurately. For this reason saturation on the Michaelis curve was not attained. For the same reason we could not determine the exact values of K_m and $V_{\max}$. From approximate calculations the value of K_m lay somewhere between 1 and $5\ \mu\text{M}$ and $V_{\max}$ was about $0.8\ \text{nmol min}^{-1} (\text{mg dry wt})^{-1}$.

As follows from Table 2, the system responsible for lysine entry was rather specific. It recognized only close structural derivatives of the amino acid. The presence of both amino groups seemed to be required for recognition. A modification of either of them (*N* α -acetyllysine, *N* ϵ -formyllysine) made the compound unable to compete with lysine for entry, while

Table 2. Transport of [^{14}C]lysine by *C. glutamicum* ATCC 13032 in the presence of structural analogues

[^{14}C]Lysine (1 μM) was present in all the experiments. δ -Hydroxylysine was a mixture of DL- and DL-allo-forms; except for D-lysine the other compounds were L-forms. The initial rate of [^{14}C]lysine entry (equivalent to 100%) was $0.06 \text{ nmol min}^{-1} (\text{mg dry wt})^{-1}$.

Analogue (40 μM)	Initial rate of entry (%)
None	100
L-Lysine	3.5
S-2-Aminoethyl-L-cysteine	15
Lysine hydroxamate	56
Spermine	56
Arginine	56
Ornithine	67
Lysine methyl ester	76
N α -Acetyllysine	80
N ϵ -Formyllysine	98
δ -Hydroxylysine	98
Diaminopimelinic acid	100
D-Lysine	100

Table 3. Effect of various substances on lysine transport and TPMP $^{+}$ accumulation by *C. glutamicum* ATCC 13032

Substance	Concn (mM)	Lysine entry (%)	Lysine retained (%)	TPMP $^{+}$ accumulation (%)
None	—	100	100	100
KCN	1	150	97	44
Sodium azide	1	108	98	ND
Rotenone	0.01	103	97	ND
DCCD	0.1	120	100	338
CCCP	0.01	15	100	11
PCP	0.1	17	103	14
Nigericin	0.005	34	98	86
Monensin	0.04	38	85	80
Nigericin + KCl	0.005 + 0.01	22	ND	ND
TPP $^{+}$	0.1	49	99	18
Valinomycin + KCl	0.005 + 0.01	25	100	32
KCl	1	81	99	ND
NaCl	1	89	94	ND

ND, Not determined.

substitutions in the carboxyl moiety (lysine hydroxamate) only partially reduced the affinity of the carrier for the derivative.

Experiments on lysine transport as a function of pH (Fig. 2) indicated that both the initial rate of uptake and the equilibrium level of accumulation appreciably increased with increasing pH up to 9.5–9.7, and then declined at higher pH values. There was no lysine accumulation whatsoever at $< \text{pH } 5$. A similar result was obtained in a study of lysine transport in *Rickettsia prowazekii* (Zahorchak & Winkler, 1983). Measurements of $\Delta\psi$ at different pH values and of the lysine potential ($\Delta\mu_{\text{lys}}$) led these authors to the conclusion that the increase in lysine uptake correlated with an increase of $\Delta\psi$. We also measured $\Delta\psi$ as a function of pH and found that it increased from 180 mV at pH 7.3 to 215 mV at pH 9.4. Meanwhile, the initial rate of [^{14}C]lysine uptake (Fig. 2) and the steady state level of accumulation (data not shown) increased three or fourfold. However, the data shown in Table 3 imply that a correlation between TPMP $^{+}$ accumulation and lysine transport existed only for exposure to permeant cations (TPP $^{+}$), valinomycin and uncouplers (CCCP, PCP). However, treatment of cells with electroneutral

Fig. 3

Fig. 3. Exchange of intra- and extracellular lysine in *C. glutamicum* 13032. The suspension of cells was divided into two portions: to one portion 0.9 μM -unlabelled lysine + 0.1 μM - $[^{14}\text{C}]$ lysine were added (●); the other portion was preincubated for 30 min with 0.9 μM -unlabelled lysine and then 0.1 μM - $[^{14}\text{C}]$ lysine was added (▲).

Fig. 4

Fig. 4. Effect of exogenous lysine (0.1 mM) on efflux of labelled lysine from *C. glutamicum* 13032 (●), strain 95 (■) and strain 10 (▲). ○, □, △, Efflux of labelled lysine from the same strains in a medium without lysine.

ionophore antibiotics (nigericin, monensin) partially suppressed lysine transport while the TPMP⁺ accumulation level remained virtually unchanged. Moreover, the drop of $\Delta\psi$ caused by uncouplers did not cause the release of intracellular lysine, a fact that is at variance with the idea of a passive distribution of lysine according to the electric potential gradient. It may be worth noting that inhibitors of ATPase (DCCD, sodium azide) or of respiration (cyanide, rotenone) and K⁺ or Na⁺ ions did not affect lysine uptake and efflux.

An unexpected finding was the weak temperature dependency of lysine transport. The Q_{10} value of the process was 1.6, which is close to the Q_{10} of diffusion.

As mentioned above, the $[^{14}\text{C}]$ lysine uptake *vs* incubation-time curve reached a plateau (Fig. 1). Since the cells already had a significant pool of unlabelled lysine when the label was added, the accumulation curve pattern observed could be thought to result from an interaction of two processes: entry of ^{14}C -labelled molecules and exit of labelled and unlabelled molecules.

The following facts support this suggestion: (i) the initial rate of $[^{14}\text{C}]$ lysine uptake was the same in cells treated with gramicidin S (no lysine pool) and in untreated cells; (ii) Fig. 3 shows that bacteria at equilibrium were still capable of taking up the label. It followed that the steady-state level was a result of exchange between extra- and intracellular lysine, mediated by the carrier. This notion was also supported by the fact that exogenous lysine could replace intracellular lysine (Fig. 4). However, cells loaded with $[^{14}\text{C}]$ lysine and placed in a lysine-free medium showed almost no lysine efflux (Fig. 4); no lysine efflux occurred when cells were placed in buffers of pH 9.7 or 5.5. Apparently, the switching on of lysine efflux requires a triggering of lysine entry. This idea was supported by the following observations: (i) lysine efflux was induced by adding lysine or its close structural derivatives to the cells but not by other amino acids; (ii) inhibition of lysine uptake at pH 5 stopped the exchange; (iii) the rate of lysine efflux dropped in the presence of inhibitors of lysine transport, e.g. CCCP. Both the uptake and the exchange diffusion of lysine in *C. glutamicum* appeared to be mediated by one and the same system.

Table 1 shows the functioning of lysine transport in various mutants. All analogue-resistant mutants (both spontaneous, selected for AEC resistance and unable to produce lysine, and obtained after mutagenization and selected for maximum production) had an altered system of lysine transport: their initial rate of lysine uptake was reduced and the increase in uptake at

Fig. 5

Fig. 6

Fig. 5. Effect of NaCl (2 M) on lysine efflux from *C. glutamicum* 13032 (●), strain 10 (▲) and strain S11 (■). ○, □, △, Efflux from untreated cells of the same strains.

Fig. 6. Effect of polymixin M (●) at 25 μ M, gramicidin S (▲) at 10 μ M and Triton X-100 (■) at 0.5% on lysine efflux from *C. glutamicum* 13032. ○, Efflux of labelled lysine from the same strain in a medium without additions.

pH 9.5 was absent (or suppressed). At the same time, a homoserine-dependent, AEC-sensitive lysine producer (strain 95) appeared to have normal transport activity. This suggested that the systems of lysine transport and excretion were not directly related to each other.

Lysine excretion from the bacteria

Obviously, the exchange mechanism that we found in wild-type bacteria could not account for the accumulation of lysine in the growth medium of producer strains. It might be suggested therefore that mutations changed the transport system of producer cells in such a way that they became able to excrete lysine in the absence of exogenous lysine. However, the data presented in Fig. 4 indicated that this was not the case. Lysine efflux from producer strain 95 (Table 1), which retained its transport activity, could only occur by the exchange of extra- and intracellular lysine, as is also the case for its wild-type parent. The question arose by what means other than the transport system could the producer cells excrete lysine. We supposed that our bacteria might possess channels that opened in response to accumulation of excess lysine in the cytoplasm. It was possible to simulate such a situation by suddenly increasing the osmotic pressure in the incubation medium. If a suspension of cells loaded with [¹⁴C]lysine was placed in a 2 M solution of KCl or NaCl, the cells should lose water, thus increasing the concentration of various metabolites, including lysine. If the lysine excretion system was indeed triggered by a certain threshold concentration of amino acid, we would be able to detect its efflux from the cells. Addition of 2 M-NaCl to a suspension of cells was followed by a rapid efflux of lysine which ended in less than a minute (Fig. 5). Accumulation of lysine was completely suppressed under these conditions. However, if a lower concentration (0.5 M) of salt was used, a small efflux was obtained after exposure of the cells first to the salt and then to exogenous lysine (Table 4). This suggested independent functioning of the lysine carrier and the lysine channels. The following facts might give support to this idea: (i) the sensitivity of the excretion system to NaCl increased with culture age while the transport system activity decreased; (ii) the NaCl-induced efflux was insensitive to CCCP.

NaCl did not cause extensive changes in membrane permeability. This was shown by the

Table 4. *Effect of salts and lysine on excretion of [14 C]lysine from C. glutamicum ATCC 13032*

Additions	Percentage lysine excretion*
None	0
NaCl (0.5 M)	20
NaCl (0.5 M) + lysine (0.1 mM)	33
KCl (0.5 M)	15
KCl (0.5 M) + lysine (0.1 mM)	49.5

* Ratio of excreted lysine to retained lysine.

following experiment. Cell proteins were labelled with [14 C]lysine: the amount of label in cell proteins was the same before and after treatment of cells with 2 M-NaCl.

Since the cellular permeability for glutamate in *C. glutamicum* and for 5'-inosinic acid in *B. ammoniagenes* can be increased by treatment with detergents, fatty acids or penicillin (Demain & Birnbaum, 1968; Furuya *et al.*, 1969; Teshiba & Furuya, 1983), we did direct experiments to check whether membrane-active agents could affect [14 C]lysine exit from *C. glutamicum* cells. The experiments indicated that detergents (gramicidin S, Triton X-100) and polymixin M, which interacts with membrane lipids, did induce the opening of hypothetical lysine channels (Fig. 6). Mutants were as sensitive to the above agents as wild-type cells. Lysis of cells was not observed after the detergent treatment.

DISCUSSION

Lysine uptake by C. glutamicum

The transport of various substances in bacteria is accomplished by means of carrier proteins which are specific for individual compounds and their structural analogues (Boos, 1974). In the majority of cases this is an active, energy-requiring process which results in a marked gradient of substrate concentration between the cytoplasm and environment. Some of the bacterial transport systems utilize the energy of the proton transmembrane electrochemical potential ($\Delta\bar{\mu}_{H^+}$) which has two components, electrical ($\Delta\psi$) and osmotic (ΔpH).

Hamilton (1975) proposed the chemiosmotic model of transport based on the ideas of Mitchell. In particular, the model implied that cations, e.g. basic amino acids, are transported by a uniport, an electrogenic process which occurs in response to a $\Delta\psi$, which is negative inside the cell. Subsequent studies confirmed this model for a number of substances. Lysine, for instance, which is positively charged at a physiological pH, is indeed distributed according to $\Delta\psi$ in *Staphylococcus aureus* and *R. prowazekii* (Niven & Hamilton, 1973, 1974; Zahorchack & Winkler, 1983).

However, Kaback and co-workers (Ramos & Kaback, 1977; Robertson *et al.*, 1980) showed that both $\Delta\psi$ and ΔpH influence the steady-state level of lysine accumulation and the kinetics of lysine transport in membrane vesicles of *E. coli*. These authors consider that 'carrier molecules must play a dynamic role in modulating the response of given transport substrate to the electrochemical proton gradient'. Also, the existence of lysine producer cells does not fully correspond to Hamilton's model: it is unclear how bacteria could export from their cytoplasm a positively charged substance against the electric potential gradient. Lysine exit may occur by cation/lysine antiport, but we failed to stimulate efflux of intracellular lysine by addition of Na^+ or K^+ (Table 3).

The present study indicates that *C. glutamicum* possesses a highly specific system of active lysine transport, the most important features of which are as follows. (i) The system acts asymmetrically, i.e. the substrate moves into the cell but not outwards, in spite of a high concentration inside. However, if lysine is present on both sides of the membrane, exchange diffusion may occur (Figs 3 and 4). The carrier, apparently, has no affinity for the inner substrate until it

binds substrate on the outside. (ii) The maximum rate of transfer and the intracellular accumulation level are observed at a pH close to the isoelectric point for lysine (pH 9.74).

Let us consider the latter fact. Lysine may exist in four forms with charges of +2, +1, 0 and -1. The pK values for the α -carboxyl, α -amino and ε -amino groups (2.18, 8.95 and 10.53, respectively) may be used to calculate the content of uncharged lysine as a function of pH. The results of such calculations by means of the Henderson-Hasselbalch equation ($\text{pH} = \text{pK}_{\text{a}} + \log \text{base/acid}$) are given in Fig. 2. The peak of transport activity corresponds to the maximum content of lysine in the form of a zwitterion. If the active site of the carrier seems to have the greatest affinity for lysine zwitterion rather than cation we suggest an alternative to Hamilton's hypothesis, i.e. that lysine translocation is accomplished in symport with H^+ by means of both components $-\Delta\psi$ and ΔpH of the electrochemical potential $\Delta\bar{\mu}_{\text{H}^+}$. If this is the case, it becomes clear why lysine accumulation is not always correlated with $\Delta\psi$.

At present, however, we cannot discriminate between the two possible mechanisms of lysine transport. We cannot discount the possibility, for example, that lysine is retained in the cytoplasm by means of a Donnan potential and that due to this lysine efflux does not occur in the presence of uncouplers.

All the AEC-resistant mutants had lowered lysine transport rates (Table 1). The acquisition of AEC resistance seems to be accompanied by a drop in the carrier activity. A transport mutation alone does not lead to changes in the intra- or extracellular lysine levels (e.g. mutants S11 and S21). Hence the ability to hyperproduce lysine is due primarily to disturbances in regulation of its biosynthesis. A combination of the latter type of mutation with a transport mutation may somewhat increase the lysine production level owing to the absence of re-accumulation of excreted lysine.

Excretion of lysine by *C. glutamicum*

Our most unexpected result was that resting producer cells, like the wild-type bacteria, did not transport their intracellular lysine outwards; their transport system could maintain only an equivalent exchange of intra- and extracellular lysine. We thought that the bacteria might excrete lysine through channels whose opening is triggered by the lysine concentration surpassing a certain threshold level, as the producer cells had unbalanced synthesis of the amino acid. Having released excess lysine, the channel would close to retain the amount of amino acid necessary for the cell's metabolism. The possible existence of such channels was demonstrated in experiments in which the osmotic pressure of the medium was rapidly increased (Fig. 5). The observed efflux of lysine was due to a rapid rise in intracellular lysine concentration since osmotic stress is known to be followed by loss of water from the cells (Griffin, 1981). The channel properties seem to depend on the cytoplasmic membrane structure, and subsequent experiments demonstrated opening of the hypothetical channels after exposure to membrane-active agents (Fig. 6). The channel permeability may also depend on the fatty acid composition of the membrane. The result obtained by Hänel *et al.* (1981) may be interpreted in terms of our hypothesis. They found the following effects of oxygen limitation on a *C. glutamicum* lysine producer: (i) the ratio of saturated to unsaturated fatty acids in the membrane was altered; (ii) the extracellular lysine concentration decreased while the intracellular concentration increased.

The lysine channels seem to play the role of safety valves in wild-type cells; they open in response to emergency situations that result in the lysine level rising. There are examples of situations when the increase of intracellular metabolite concentration leads to the efflux of this substance into the medium. Thus suppression of γ -glutamyl transpeptidase in *Proteus mirabilis* and some other bacteria resulted in an increased intracellular glutathione content and an efflux of this metabolite (Nakayama *et al.*, 1984). In *E. coli*, inhibition of RNA synthesis was accompanied by a 100-fold increase in glutamate secretion (Broda, 1968). According to the author, the increase of membrane permeability under these conditions was due to accumulation of metabolites that could not be utilized in biosynthesis.

The nature, mechanism of action or specificity of the hypothetical channels of *C. glutamicum* are far from being understood at present. They should be the subject of further investigation.

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