Lysine secretion by wild-type *Corynebacterium glutamicum* triggered by dipeptide uptake

ANDREAS ERDMANN,* BRITA WEIL and REINHARD KRÄMER

Institut für Biotechnologie 1, Forschungszentrum Jülich, Postfach 1913, D-52425 Jülich, Germany

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In *Corynebacterium glutamicum* peptide uptake increases the internal concentration of amino acids and thus triggers amino acid secretion. The peptide uptake system is stimulated by a factor of two in cells grown on pure peptone medium in comparison to peptone media with additional carbon sources. Uptake depends on the proton-motive force and shows a broad substrate spectrum. Peptide uptake is characterized by a K_m of about 230 μM and a $V_{\max}$ of 12 $\text{nmol min}^{-1} (\text{mg dry wt})^{-1}$ for the peptide lysyl-alanine (Lys-Ala). Lysine secretion in the wild-type of *C. glutamicum* does not show Michaelis–Menten-type kinetics as reported for the producing strains DG 52-5 and MH 20-22B. The secretion of lysine depends on the composition of the medium in which the cells were grown prior to the initiation of secretion by peptide uptake. The lack of secretion activity when the cells are shifted to peptone medium in the presence of chloramphenicol indicates that protein synthesis is necessary for this regulatory process.

Introduction

Corynebacterium glutamicum and related organisms are widely used for the industrial production of amino acids, especially glutamate and lysine. The producing strains are mostly derived from the wild-type of *C. glutamicum* by classical mutagenesis (Nakayama *et al.*, 1966) and possess an aspartate kinase insensitive to feedback regulation. This enzyme mainly controls lysine biosynthesis in the wild-type by concerted feedback inhibition of lysine plus threonine.

Recently, it was demonstrated that in the producing strain DG 52-5 lysine is secreted together with 2 OH^- by a cotransport carrier showing Michaelis–Menten-type kinetics (Bröer & Krämer, 1991*a*). Such an export carrier was also expected in the wild-type of *C. glutamicum* but because of the low internal lysine concentration no secretion activity was detected.

In this connection, the question arises of the physiological significance of a lysine export carrier. *C. glutamicum* can certainly use complex sources, such as proteins and their degradation products, for nutrition. Peptides may fulfil essential amino acid requirements or can serve as the sole nitrogen and/or carbon source. Such processes are well established for Gram-negative bacteria, e.g. *E. coli*, where three genetically different peptide uptake systems can be found (Higgins & Gibson,

1986). Two of these peptide transport systems belong to the periplasmic or binding-protein transport family (Cowell, 1974). Among Gram-positive bacteria peptide uptake is well characterized in the genus *Lactococcus* (Smid *et al.*, 1989*a, b*). *Lactococcus lactis* possesses a secondary di-tripeptide uptake system dependent on the proton-motive force, which is essential for growth on casein as the sole nitrogen source (Smid *et al.*, 1989*b*). Recently, a second energy-requiring peptide uptake system transporting oligopeptides was described in *L. lactis* (Kunji *et al.*, 1993).

Since *C. glutamicum* is not able to metabolize lysine (Nakayama, 1985), the existence of a specific export carrier may serve to keep the internal lysine concentration at a certain level during growth on complex media. Another possibility of avoiding increased internal lysine concentration would be selection at the level of peptide/amino acid uptake. Thus studies concerning the uptake of lysine-containing peptides should allow the investigation of lysine secretion in the wild-type of *C. glutamicum*.

Methods

Growth of organism. The strain used was *C. glutamicum* ATCC 13032 (wild-type). Cells were grown under aerobic conditions on a rotary shaker (130 r.p.m.) at 30 °C in a complex medium (g l^{-1}): peptone, 15.6; yeast extract, 2.8; NaCl, 5.6; biotin, 0.0002; pH 7.0. The following substances were used as additional carbon sources: 5% (w/v) glucose; 100 mM-sodium glutamate; or 100 mM-sodium acetate. In some experiments cells were grown in minimal medium (g l^{-1}): $(\text{NH}_4)_2\text{SO}_4$, 5;

* Author for correspondence. Tel. +49 2461 615556; fax +49 2461 612710.

urea, 5; KH_2PO_4 , 2; K_2HPO_4 , 2; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.01; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01; biotin, 0.0002; glucose, 50; pH 7.0. Cell mass was determined by measuring the optical density (LKB spectrophotometer 4040 Nova Spec II) at 600 nm (OD_{600}); $\text{OD}_{600} = 1$ corresponds to 0.32 mg dry wt ml^{-1} .

Determination of peptide uptake and lysine secretion. After growing the cells overnight, 5 ml of the culture were either inoculated into a medium of the same composition (100 ml) or containing a different carbon source. If the carbon source had been changed the cells were washed twice before inoculation by centrifugation (4000 r.p.m., 4 °C, 10 min) in a sterile buffer containing 50 mM-MOPS, 10 mM-NaCl, 10 mM-KCl, pH 7.5. The same washing procedure was used to prepare the cells prior to the peptide uptake experiment after growth for 2.5 h or 5 h to the exponential phase.

Peptide uptake and lysine secretion was initiated by adding the cells to prewarmed (25 °C) buffer containing 1 mM-peptide, 10 mM-sodium glutamate or 20 mM-glucose, 50 mM-MOPS, 10 mM-NaCl, 10 mM-KCl, pH 7.5 at an initial OD_{600} of 7. In most experiments, glutamate was used as the carbon source because of possible repression by glucose. In this short-term fermentation, the cells were stirred rapidly by a magnetic stirrer at 500 r.p.m. to ensure a sufficient oxygen supply.

In order to determine the intra- and extracellular concentrations of metabolites (peptide, glutamate, lysine, alanine, glucose) cells and external solution were separated by silicone oil centrifugation (Klingenberg & Pfaff, 1967). The cell-free supernatant was used directly for metabolite determination by reversed-phase HPLC (HP 1090 M, Hewlett-Packard) employing precolumn derivatization with *ortho*-phthalaldehyde reagent (Pierce) and subsequent fluorescence detection. In the case of glucose an enzymic determination was used (Boehringer) as described by Kunst *et al.* (1983).

Preparation of cell extracts for determination of intracellular amino acids. By means of centrifugation, 100 μl of cell suspension was sedimented through 65 μl silicone oil (density 1.03 g cm^{-3}) into 30 μl perchloric acid (20%, v/v). The tube was cut within the upper layer of oil, and the denatured cells were resuspended in 45 μl water together with 10 μl of silicone oil with a density of 1.07 g cm^{-3} to absorb membrane material. After breaking up the cells by sonication, neutralizing the extract by addition of 25 μl buffer (5 M-KOH, 1 M-triethanolamine) and removing precipitated protein and cell wall material by centrifugation, the extract was used for amino acid analysis as described above (see also Ebbighausen *et al.*, 1989).

Internal ATP concentration. The measurement of intracellular ATP was carried out by direct addition of the cell suspension (20 μl) to dimethylsulphoxide (180 μl) (Duperray *et al.*, 1992). Samples were further diluted (1:5) with cold distilled water and kept at -20 °C until determination of ATP. This was done by using the firefly luciferase assay (XP 2000, Skan AG) with an internal ATP standard for calibration (Schimz *et al.*, 1981).

Cytoplasmic volume and membrane potential. Determination of cytoplasmic volume is based on comparison of the space occupied by a permeable (water) and an impermeable marker (taurine). The procedure is described in detail elsewhere (Bröer & Krämer, 1990). Measurement of cells grown in complex media resulted in a value of 1.8 μl (mg dry wt) $^{-1}$. The distribution of the lipophilic cation [^{14}C]tetraphenylphosphonium was measured to determine the membrane potential. Different values of membrane potential were achieved by addition of 10 μM -valinomycin to cells suspended in solutions with various K^+ concentrations. The values of membrane potential measured were corrected for nonspecific binding according to the model of Zaritsky *et al.* (1981). The application of this method for *C. glutamicum* has been described elsewhere (Ebbighausen *et al.*, 1989).

Calculations. Initial velocities were calculated by linear regression of the first (linear) part of the plot of external concentration ($\mu\text{mol l}^{-1}$)

vs. time (min). These values were then recalculated by introducing the initial OD_{600} to obtain values with units of nmol min^{-1} (mg dry wt) $^{-1}$. A negative sign for velocities indicates uptake of the substance specified, a positive (no) sign represents secretion. Values are means $\pm$ 1 SD. The uptake velocity of Lys-Ala in Fig. 3 was determined by calculating the tangent to the curve of extracellular peptide concentration vs. time. These values were recalculated with reference to the actual OD_{600} and were related to the external peptide concentration measured at the same time in an Eadie-Hofstee plot.

Chemicals. [^{14}C]Tetraphenylphosphonium bromide (TPP^+) was obtained from NEN/DuPont. Biochemicals were supplied by Boehringer. All other chemicals were of analytical grade and obtained from Merck and Sigma. All peptides were purchased from Bachem (Heidelberg).

Results

Physiological significance of lysine secretion

Wild-type cells of *C. glutamicum* grown on complex medium without additional carbon source showed rapid uptake of the peptide lysyl-alanine (Lys-Ala). Subsequently, an increase in internal concentrations of lysine and alanine due to hydrolysis by intracellular peptidase was observed. As a consequence, lysine and alanine were secreted into the medium (Fig. 1). After consumption of the peptide, the internal lysine concentration decreased until it reached its initial value of about 10 mM (Fig. 1a). The sum of external and internal lysine and the amount of lysine contained in the peptide was constant over the entire time period. This indicated that no metabolism of lysine took place under these conditions and that internal hydrolysis of the peptide had to be very fast.

In contrast, both the external and internal alanine concentration decreased after consumption of the peptide. Alanine secreted into the medium was afterwards taken up again, and together with the disappearance of internal alanine, this indicated that alanine was almost entirely metabolized (Fig. 1b).

In order to test whether alanine was sufficient to support growth of *C. glutamicum* and thus whether the organism is able to utilize peptide as the sole nitrogen source, the experiment shown in Fig. 2 was performed. The organism was able to grow using Lys-Ala as sole nitrogen source and 50 mM-glucose as carbon source, in a buffer containing 50 mM-MOPS, 10 mM-NaCl, 10 mM-KCl and biotin (0.2 mg l^{-1}). Growth, however, could be achieved only if the cells were grown on complex medium to the exponential phase prior to the peptide utilization experiment. Growth on peptide as sole nitrogen and carbon source was not observed (data not shown). The reason for the inhibition of growth after 20 h remains unclear since neither the peptide nor the glucose was entirely consumed. High internal concentrations of metabolites (e.g. lysine) might be one factor inhibiting growth.

Fig. 1. Uptake of 1 mM-Lys-Ala (■) by *C. glutamicum* grown on complex (peptone) medium and resulting changes in the extra- (●) and intracellular (▲) concentration of lysine (a) and extra- (○) and intracellular (△) alanine (b). Transport was measured during short-term fermentation as described in Methods. MOPS buffer was supplemented by 10 mM-glutamate as carbon source. Each data point represents the mean of six independent experiments.

Fig. 2. Growth of *C. glutamicum* (▼) on Lys-Ala as sole nitrogen source. Consumption of Lys-Ala (■) and glucose (▲) (carbon source) are indicated together with secretion of lysine (●).

Characterization of peptide uptake

Since utilization of the peptide for growth could be observed only if cells were previously grown without glucose, peptide uptake may depend on culture con-

Fig. 3. Uptake of Lys-Ala. Inset: Eadie-Hofstee-plot of uptake velocity, V [$\text{nmol min}^{-1} (\text{mg dry wt})^{-1}$] vs. $V/\text{external concentration of peptide}$ [$\text{nmol} (\text{mg dry wt})^{-1} \text{min}^{-1} \mu\text{M}^{-1}$]. K_m ($230 \mu\text{M}$) and $V_{\max}$ [$12.5 \text{ nmol min}^{-1} (\text{mg dry wt})^{-1}$] values were calculated as described in Methods. Each data point represents the mean of two independent experiments.

ditions. Any addition, independent of the carbon source (100 mM-glutamate, 100 mM-acetate or 5%, w/v, glucose), to the complex (peptone) medium reduced the initial velocity of peptide uptake by about 50% compared to media without any addition [initial velocity of Lys-Ala uptake: $-11.4 \pm 3.5 \text{ nmol min}^{-1} (\text{mg dry wt})^{-1}$]. The lowest transport activity was found in cells grown in the minimal medium: $4.0 \pm 0.8 \text{ nmol min}^{-1} (\text{mg dry wt})^{-1}$.

Specificity. In order to increase the internal lysine concentration effectively the specificity of the peptide uptake system was investigated. The decrease of extracellular peptide with time occurred in all experiments similar to that shown in Fig. 1(a). Besides the uptake of Lys-Ala, four other lysine-containing peptides were used: Ile-Lys, Lys-Ile, Lys-Glu and Lys-Lys-Lys. The initial uptake velocities ranged from $-6.4 \text{ nmol min}^{-1} (\text{mg dry wt})^{-1}$ for Lys-Lys-Lys to $-2.7 \text{ nmol min}^{-1} (\text{mg dry wt})^{-1}$ for Lys-Glu. Additionally, the transport of five other alanine-, valine-, isoleucine- or leucine-containing peptides was measured (S. Zittrich, personal communication). Of the ten peptides investigated, Lys-Ala was taken up most rapidly (see above) whereas Ile-Val showed the lowest transport velocity, $1.2 \text{ nmol min}^{-1} (\text{mg dry wt})^{-1}$. The absolute values of peptide uptake are in the range reported in the literature for various organisms (Payne & Gilvarg, 1978; Nisbet & Payne, 1982; Smid *et al.*, 1989a; Smid, 1991).

Affinity. The $V_{\max}$ and K_m of peptide uptake were calculated from the decrease in the extracellular concentration of Lys-Ala (Fig. 3). Due to the limited sensitivity of non-radioactive uptake measurements of peptides (HPLC), these values were determined using the deviations from linearity of uptake at low peptide

Fig. 4. (a) Dependence of membrane potential (■) measured by TPP⁺ distribution and internal ATP concentration (◇) on external K⁺ concentration in the presence of 20 μ M-valinomycin. (b) Relation between membrane potential obtained as described in (a) and uptake velocity of Lys-Ala. Peptide uptake was measured during short-term fermentation in MOPS buffer supplemented by 5% (w/v) glucose.

concentrations (see Methods). The $V_{\max}$ was calculated to be 12.53 ± 0.96 nmol min⁻¹ (mg dry wt)⁻¹ for Lys-Ala and the K_m value was 230 ± 53 μ M. This peptide was used for further experiments.

Mechanism. In order to obtain insight into the mechanism of peptide uptake in *C. glutamicum*, the influence of ATP and membrane potential on peptide uptake was tested. The protonophore CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) inhibited uptake of Lys-Ala by 78% at a concentration of 15 μ M and by 83% at 200 μ M. The strongest inhibition (95%) was obtained by the combination of 2 μ M-valinomycin and 0.2 μ M-nigericin in the presence of 300 mM-KCl. Since under such conditions (buffer supplemented by 5% glucose) the ATP concentration was also decreased in comparison to the control without ionophore, the influence of these two parameters on peptide uptake was investigated in more detail. Fig. 4(a) shows the dependence of the membrane potential on the external K⁺

concentration in the presence of 20 μ M-valinomycin. Although being somewhat reduced, the ATP concentration does not depend on the external K⁺ concentration. The linear relation between membrane potential and the logarithm of the uptake rate (Fig. 4b) serves as additional evidence for the mechanism of secondary transport, at least for the peptide Lys-Ala.

Characterization of peptide-triggered lysine secretion

Peptide uptake and subsequent hydrolysis increased the internal lysine concentration in the wild-type of *C. glutamicum* (Fig. 1a). As a consequence lysine was secreted. This was true of all conditions investigated. The initial efflux velocity was dependent on the composition of the medium in which the cells were grown prior to peptide uptake (Fig. 5a). Growth in peptone medium without additional carbon source resulted in a velocity of 6.8 ± 0.4 nmol min⁻¹ (mg dry wt)⁻¹ ($n = 6$). This value was at least twice as high as the values found for media with an additional carbon source ($n \geq 3$). These were: in peptone with 5% (w/v) glucose, 1.6 ± 0.6 nmol min⁻¹ (mg dry wt)⁻¹; in peptone with 100 mM-acetate, 2.5 ± 1.9 nmol min⁻¹ (mg dry wt)⁻¹; in peptone with 100 mM-glutamate, 2.3 ± 1.0 nmol min⁻¹ (mg dry wt)⁻¹; in mineral medium with 5% (w/v) glucose, 3.0 ± 0.4 nmol min⁻¹ (mg dry wt)⁻¹. Although the values of initial efflux velocity obtained after growth in media with various additional carbon sources were not significantly different, Fig. 5(b) shows that further changes in the transport process had to be taken into account. After consumption of the peptide Lys-Ala (indicated by dotted arrows), the ability to lower the internal lysine concentration differed in the various kinds of cells. Cells grown in peptone medium with 5% (w/v) glucose maintained a high internal lysine concentration (Fig. 5b) for at least 6 h (data not shown). The results of Fig. 5 indicate that the initial velocity of secretion is not sufficient to characterize these transport phenomena, but the time course of internal lysine concentration also had to be studied. Since the data of Fig. 5 could be explained by a regulatory process, we investigated whether this process could be observed by changing the carbon source of the media for a short period before testing for peptide uptake.

After growth overnight in peptone medium, with or without 5% (w/v) glucose, the cells were transferred to peptone medium without additional carbon source for 2.5 h. Fig. 6 shows that cells grown solely in peptone medium exhibited the highest initial velocity of lysine secretion and a relatively small and transient increase of the internal lysine. In comparison to the data in Fig. 5, cells grown overnight in the presence of glucose could be stimulated to secrete lysine by subsequent growth in pure

Fig. 5

Fig. 6

Fig. 5. Lysine secretion (*a*, external concn; *b*, internal concn) by *C. glutamicum* triggered by dipeptide uptake. Cells were grown on complex (peptone) medium (●), on peptone with 100 mM-glutamate (▼), on peptone with 100 mM-acetate (▲), and on peptone with 5% glucose (■). Dotted arrows in (*b*) indicate the point of complete peptide consumption. Peptide uptake was followed during short-term fermentation in MOPS buffer supplemented by 10 mM-glutamate. Each data point represents the mean of at least three independent experiments.

Fig. 6. Induction of lysine secretion (*a*, external concn; *b*, internal concn) in *C. glutamicum* by changing the growth medium. Cells were either grown on peptone medium alone (●) during the whole period or the growth medium was shifted from peptone with 5% glucose (overnight) to peptone alone for 2.5 h (▲). ■, Growth medium changed as above but 50 µg chloramphenicol ml⁻¹ was added to the peptone medium without glucose. Peptide uptake was followed during short-term fermentation in MOPS buffer supplemented by 20 mM-glucose.

peptone medium for 2.5 h. The secretion activity in this case did not reach the maximal value. The observed stimulation could be prevented in these cells by adding the protein synthesis inhibitor chloramphenicol (50 µg ml⁻¹) after shifting to peptone medium. In the experiments of Fig. 6, peptide uptake was investigated in a buffer supplemented with glucose, since the uptake of glutamate is also dependent on the medium composition (Krämer *et al.*, 1990).

In order to identify a possible regulatory compound responsible for the observed stimulation, the internal amino acid composition of cells grown in peptone medium with or without 5% (w/v) glucose was analysed. We detected significant differences in the concentrations of internal amino acids (e.g. glutamate, glycine, alanine, serine, arginine). In particular, the increased concentration of lysine (14.4 mM) in medium without glucose in comparison to medium with glucose (8.6 mM) should be considered.

Discussion

A specific lysine secretion system was previously characterized in producing strains of *C. glutamicum* (Bröer & Krämer, 1991*a, b*). It was suggested that this secretion carrier serves to regulate the internal lysine concentration under conditions where complex substances are the main nutrients. Uptake of peptides and consecutive efflux of amino acids has also been shown to occur in lactococci (Perry & Abraham, 1979; Nisbet & Payne, 1982). Possible interference by the presence of extracellular peptidases, as discussed in connection with peptide utilization (Smid *et al.*, 1989*a*), was ruled out for *C. glutamicum*. After drastic reduction of the membrane potential by CCCP or valinomycin in the presence of high external K⁺ concentrations the external peptide concentration remained more or less constant for a reasonable period of time (data not shown). Since the uptake of lysine in *C. glutamicum* is very low (Bröer &

Krämer, 1990) this flux also did not need to be considered as a possible artefact here.

Using the dipeptide Lys-Ala, we demonstrated that *C. glutamicum* was able to grow on complex nutrients as the sole nitrogen source (Fig. 2). Similar results were reported by Brecher *et al.* (1992) concerning the growth of *E. coli* on degradation products of chymotrypsin, where the peptides support growth most effectively as the nitrogen source and less as the carbon source. Utilization of peptides by *C. glutamicum* did occur even if lysine, which cannot be metabolized (Nakayama, 1985), was present in the peptide. The theoretical possibility of avoiding an increase in internal lysine by selection at the level of peptide uptake did not take place. Therefore, it is reasonable to conclude that secretion carriers serve to control the amino acid pool under physiological conditions of growth on complex nutrients.

Since we found that *C. glutamicum* utilizes peptides only in the case of growth on complex medium without glucose, an influence of the medium composition on peptide uptake and/or lysine secretion had to be considered. In *E. coli* the presence of external amino acids has no influence on the uptake of peptides containing the same amino acids (Payne & Bell, 1977). *E. coli* possesses three genetically different peptide uptake systems (Hiles *et al.*, 1987; Abouhamad *et al.*, 1991), of which only one, the tripeptide permease (Tpp), can be stimulated by external leucine as well as by anaerobiosis. *C. glutamicum* showed the highest peptide uptake rate when cells were grown on proteins and their degradation products only (peptone medium). The addition of other carbon sources reduced the uptake velocity by about 50%. Since this phenomenon was independent of the nature of the carbon source (amino acid, sugar or organic acid) it could be assumed that external peptides themselves were the reason for the observed differences. Specificity or affinity (Fig. 3) of peptide uptake by *C. glutamicum* was comparable to other systems described in the literature (Payne & Bell, 1979; Nisbet & Payne, 1982; Smid, 1991; see also Results). The existence of more than one peptide uptake system in *C. glutamicum* cannot be excluded by the results obtained, although the results of Fig. 3 indicate that the dipeptide Lys-Ala is taken up by one kinetically distinguishable transport system.

Peptide uptake systems were characterized as being binding-protein-dependent in Gram-negative as well as in Gram-positive bacteria (Van Boven & Konings, 1987; Abouhamad *et al.*, 1991; Kunji *et al.*, 1993). For Gram-positive bacteria the existence of secondary transport systems for peptides dependent on the proton-motive force as the driving force has also been reported (Payne & Nisbet, 1981; Smid *et al.*, 1989a). Data presented in Fig. 4, showing a linear relation between the logarithm of

peptide uptake rate and membrane potential, demonstrate that the observed peptide uptake in *C. glutamicum* depended on the proton-motive force rather than on ATP. The uptake rate under 'saturation' conditions at very high values of the membrane potential corresponds to initial uptake rates measured under similar conditions (MOPS buffer supplemented by glucose) without the addition of valinomycin. Such a 'saturation' effect was also described for the membrane-potential-dependent secretion of lysine in *C. glutamicum* (Bröer & Krämer, 1991b). The concomitant inhibition of lysine secretion under conditions of reduced membrane potential (data not shown) revealed that characteristics of lysine export in the wild-type of *C. glutamicum* are comparable to those shown for producing strains (Bröer & Krämer, 1991b).

In contrast to lysine-producing strains of *C. glutamicum*, which are thought to differ from the wild-type in having a feedback-insensitive aspartate kinase (Menkel *et al.*, 1989), no effective lysine secretion could be achieved for the wild-type by growth on glucose-containing minimal media (Bröer *et al.*, 1993). Therefore we used the procedure of peptide feeding to increase the internal lysine concentration in the wild-type. The results shown in Fig. 1(a) and 5(b) indicate that the concentration can be raised to at least 25 mM under all conditions investigated. This increase did not cause an appropriate secretion of lysine as would be expected from Michaelis-Menten-type kinetics, despite the fact that the values of internal lysine found after peptide uptake exceeded the internal K_m of 20 mM calculated for the producing strain DG 52-5 (Bröer & Krämer, 1991a). Non-Michaelis-Menten-type kinetics was also observed for a recombinant strain of *C. glutamicum* where the wild-type aspartate kinase was replaced by a feedback-insensitive one, leading to elevated internal lysine concentrations (Bröer *et al.*, 1993). We conclude that additional regulatory mechanisms have to be taken into account.

The results concerning dipeptide-triggered lysine secretion shown in Fig. 5 argue for a dependence of lysine secretion activity on the composition of the growth media. The initial velocity of lysine secretion was about three times higher in cells grown in peptone medium as compared to those grown with additional carbon source. The different behaviour of lysine secretion can be interpreted as a regulatory (possibly induction/derepression) phenomenon. In order to identify an appropriate effector, the internal amino acid composition was investigated in cells grown on peptone (highest secretion rate) and on peptone with glucose (lowest secretion rate). It was possible to detect significant differences between cells grown in these two media. As is known for various inducible amino acid uptake systems,

the presence or absence of the amino acid to be transported is responsible for increased transport activity (Ring, 1969; Miller & Rodwell, 1971; Quay & Oxender, 1976; Steffes *et al.*, 1992). By analogy to this and to the induction of the uptake system for branched-chain amino acids in *C. glutamicum* (Boles *et al.*, 1993), it might be assumed that the high internal lysine concentration found in cells grown in the absence of glucose (see results) serves as a stimulating effector for the regulation of lysine secretion. Additionally, the presence of glucose may repress the transport, as reported for glutamate uptake in *C. glutamicum* (Krämer *et al.*, 1990).

Both phenomena, induction and derepression, require *de novo* synthesis of protein. Thus dipeptide-triggered lysine secretion was investigated after inhibition of protein synthesis by chloramphenicol. Since the uptake of Lys-Ala was almost unchanged by this treatment (data not shown) the decrease in the secretion rate in the presence of chloramphenicol (Fig. 6a) must be caused by preventing the observed regulatory process(es) by inhibition of protein synthesis. Significant differences in the internal lysine concentration were not found in cells grown in the presence or absence of chloramphenicol (data not shown). The possibility that lysine secretion is mediated by a carrier that is cooperatively controlled by lysine and/or other unknown effectors cannot be entirely excluded by these experiments. At the molecular level, the mechanism of reduction of lysine secretion by the addition of external carbon source still remains to be studied.

In the wild-type of *C. glutamicum* lysine is secreted by a specific carrier after increasing the internal pool by dipeptide uptake. The lysine secretion system is presumably stimulated at the expression level by high internal lysine (and/or by other amino acid) concentrations. In contrast to lysine secretion in producing strains (Bröer & Krämer, 1991a), Michaelis–Menten-type kinetics could not be observed. A similar result was reported by Bröer *et al.* (1993) for a recombinant strain differing from the wild-type only in its aspartate kinase. Therefore, additional regulatory processes at the activity level concerning the secretion rate have to be taken into account. In summary, the observed regulatory processes of lysine secretion provide the wild-type of *C. glutamicum* with fast and sensitive response mechanisms to environmental changes.

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