# DIFFERENTIAL EFFECTS OF GENERAL AMINO ACID CONTROL OF LYSINE BIOSYNTHESIS ON PENICILLIN FORMATION IN STRAINS OF *PENICILLIUM CHRYSOGENUM*

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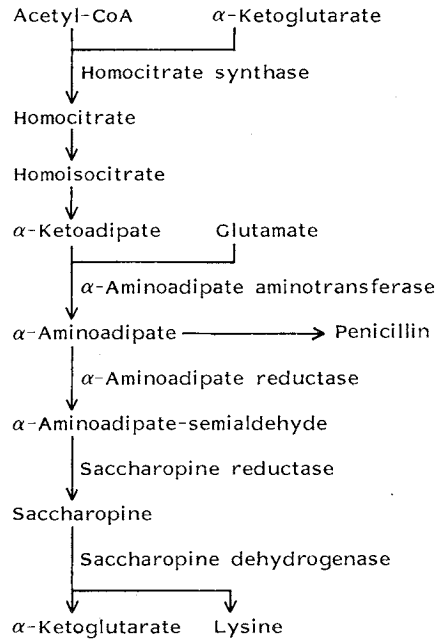
The biosynthesis of penicillin by strains of *Penicillium chrysogenum* requires a supply of the amino acids valine, cysteine and α-aminoadipic acid[1]. α-Aminoadipic acid has received most attention since it is an intermediate of lysine biosynthesis in fungi[2] and therefore forms a branch point between lysine and penicillin biosynthesis in *P. chrysogenum*[3~6]. Higher producing strains of *P. chrysogenum* were shown to contain higher intracellular α-aminoadipate pool concentrations during penicillin fermentation, suggesting that, although this amino acid is recycled during the last steps of penicillin biosynthesis[3], the pool level is an important parameter determining the flux to penicillin[6]. The mechanisms in regulation of lysine biosynthesis responsible for the different steady-state levels of α-aminoadipate are, however, not known.

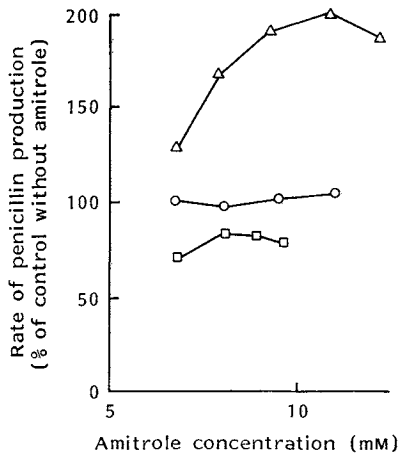
JAKLITSCH *et al.*[7] have recently shown that at the level of gene expression, lysine biosynthesis in *P. chrysogenum* is regulated mainly by general amino acid control[8], *i.e.* by derepression of certain enzymes upon starvation of at least one of several amino acids, *e.g.* histidine or arginine. In *P. chrysogenum* Q 176, saccharopine reductase (EC 1.5.1.10) and saccharopine dehydrogenase (EC 1.5.1.7) are subject to general amino acid control (Fig. 1). In the higher producing strains D6/1014/A and P2 α-aminoadipate reductase is included in this regulatory process[7]. This difference in coarse control of the enzyme catalyzing α-aminoadipate breakdown prompted us to investigate whether changes in the activity of α-aminoadipate reductase are responsible for the increased α-aminoadipate pool size in the higher producing strains during penicillin formation. We have evoked general amino acid control by the histidine analogue amitrole (3-amino-1,2,4-triazole)[9] to manipulate mycelial levels of α-aminoadipate reductase under conditions where penicillin is formed in *P. chrysogenum*.

Fig. 1. Schematic drawing of the pathway of lysine and α-aminoadipate biosynthesis in *Penicillium chrysogenum*, indicating the position of enzymes mentioned in the text.




The effect of amitrole on penicillin formation in a recently described replacement system[6] is shown in Fig. 2 for *P. chrysogenum* strains Q 176, D6/1014/A and P2: It is clearly different for each strain. Q 176 was affected not at all over the whole concentration range tested. In contrast, strain D6/1014/A displayed significant stimulation between 5 and 10 mM which corresponds to the amitrole concentration required to evoke general amino acid control[7]. On the other hand, penicillin biosynthesis was inhibited up to 30% in strain P2. The difference in behavior in D6/1014/A and P2 was unexpected since both strains exhibited elevated levels of α-aminoadipate reductase activity upon amitrole addition[7]. We have therefore measured the effect of amitrole on the intracellular concentration of α-aminoadipate, again using the replacement system; amino acids were extracted as described recently[6] and quantified by HPLC

Fig. 2. Effect of amitrole on penicillin biosynthesis in *Penicillium chrysogenum*.
△ D6/1014/A, ○ Q 176, □ P2.




Different strains are indicated in the figure. Penicillin production was carried out in a defined medium described recently[6], but omitting cycloheximide and chloramphenicol. Penicillin production rates were calculated from the linear increase in extracellular penicillin during the first 3 hours.

Table 1. Effect of amitrole (10 mM) on pool concentrations of some amino acids in mycelia of *Penicillium chrysogenum* strains[a].

| Amino acid | Strain | | |
|---|---|---|---|
| | Q 176 | D6/1014/A | P2 |
| Valine | 98 | 79 | 92 |
| Cysteine | 100 | 70 | 100 |
| α-Aminoadipate | 100 | 185 | 92 |
| Lysine | 125 | 111 | 118 |
| Arginine | 156 | 130 | 163 |
| Phenylalanine | 113 | 100 | 115 |

[a] Values are quoted as the percent of changes in the intracellular pool of the particular amino acid in medium supplemented with amitrole (10 mM) as compared to the control without amitrole. For this purpose, mycelia were taken at the beginning of incubation in the replacement medium and extracted for amino acids as described[6]. All values are means of at least three separate experiments. The standard deviation for all amino acids except α-aminoadipate was less than ±17%, but ±30% for α-aminoadipate.

following derivatization with phenylisothiocyanate[10]. This method yielded values consistent with these previously obtained by GC and ion-exchange chromatography[6]. The pool of α-aminoadipate was very low in Q 176 and was not affected by amitrole (Table 1). The pool concentration in P2 was marginally decreased; as stressed previously, the comparatively small size of the α-aminoadipate pool precludes its determination with a standard deviation less than ±30%[6]. Since the changes actually found upon addition of amitrole were below this value it is not possible to be sure whether strain P2 has a lower α-aminoadipate pool concentration. In strain D6/1014/A, however, significantly elevated α-aminoadipate pool concentrations were found in the presence of amitrole. Other amino acids related to penicillin biosynthesis (valine, cysteine) were not elevated upon amitrole addition, and therefore cannot be involved in the stimulation of penicillin formation D6/1014/A. The effect of general amino acid control could be seen in the elevated levels of arginine, but histidine levels could not be measured, since the histidine peak overlapped with an unknown compound. The concentration of lysine and phenylalanine remained fairly constant.

These results are consistent with the correlation between intracellular α-aminoadipate pool concentration and penicillin formation observed previously[6], but inconsistent with a role for α-aminoadipate reductase in the control of the α-aminoadipate pool. While it is difficult to predict whether an elevation in the activity of an enzyme catalyzing the metabolism of an intermediate will lead to an increase or decrease in the intermediate's steady state level, the disparate effects of an increase in α-aminoadipate reductase on the α-aminoadipate pool in strain D6/1014/A and P2 indicate that changes in the activity of this enzyme alone are not responsible. Changes in α-aminoadipate pool levels must therefore be the result of other fine control mechanisms in these strains of *P. chrysogenum*.

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