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EFFECT OF GLUTAMINE ON PENICILLIN FORMATION IN *PENICILLIUM CHRYSOGENUM*

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SUMMARY

Resting-cell studies in *Penicillium chrysogenum* have indicated that penicillin formation is inhibited by glutamine concentrations higher than 1 mM. Total inhibition was obtained with 10 mM glutamine. This action was neither reverted by the amino acid precursors of the antibiotic moiety nor glutamine affected the *in vitro* activity of the first enzyme of the penicillin formation pathway. The inhibition was prevented by 1 mM glutathione by mechanisms not related to limitation in the glutamine incorporation nor connected with degradation of the tripeptide.

INTRODUCTION

The participation of glutamine, not only as precursor in the synthesis of primary and secondary metabolites, but also as a key regulator in the synthesis and degradation of several compounds (Marzluf, 1981; Sanchez *et al.*, 1981), is well known. Previous studies from this laboratory have suggested that the ability of *P. chrysogenum* to produce penicillin is closely related to glutamine synthetase activity and resting-cell studies have indicated that penicillin formation is preferentially stimulated by limiting concentrations of glutamine, rather than by similar concentrations of glutamate or ammonium (Sanchez *et al.*, 1981). Under these conditions, we decided to further explore the physiological effects of higher glutamine concentrations.

MATERIALS AND METHODS

Organism and cultivation. *P. chrysogenum* NRRL 1951 was kindly supplied by the Agricultural Research Service Culture Collection, Northern Regional Research Laboratory, Peoria, IL. 61604 U.S.A. All cultures were grown for 36 h in a defined medium (DM), as previously reported (Lara *et al.*, 1982).

Resting-cell system studies. For this purpose the 36 h culture (100 ml) was harvested, washed with 2 vol distilled water and resuspended in 50 ml of suspension medium (Sanchez *et al.*, 1981). Under these conditions cells produced penicillin linearly during 36 h in the presence of cycloheximide (100 µg ml^{-1}), without any increase in dry cell weight. At desired times, penicillin was determined according to Aharonowitz and Demain (1982), using benzylpenicillin as standard.

δ(L-α-aminoadipyl)L-cysteine synthetase activity. After growth (36 h) in DM medium and further incubation (36 h) in the antibiotic formation medium (AFM) (prepared with the standard salt mixture of Jarvis and Johnson (1947), supplemented with 83 mM lactose, 3.6 mM phenylacetate and 8.5 mM NH_4Cl) at 29°C in a rotary shaker at 160 rev min^{-1} (500 ml contained in a 2800 ml Fernbach flask), cell-free extracts of *P. chrysogenum* were prepared as described by Lara *et al.* (1982). Enzyme activity was determined at 37°C in the reaction system previously reported with the modification that 2-mercaptoethanol was eliminated (Lara *et al.*, 1982). Specific activity was expressed in µkat Kg $protein^{-1}$.

Protein determination. Protein was measured by the Lowry method using bovine serum albumin as standard.

Determination of glutamine transport. For this purpose, 50 ml of the 36 h culture were harvested and resuspended in 24 ml of the suspension medium with cycloheximide (100 µg ml^{-1}) contained in a 125 ml Erlenmeyer flask. The flask was incubated at 29°C under continuous agitation (150 rev min^{-1}). After 5 min, 1 ml of L-[U-^{14}C] glutamine (0.6 µCi μmol^{-1}) was added to the system and samples were withdrawn at desired times for further analysis as previously reported (Sanchez *et al.*, 1972).

RESULTS

In a resting-cell system with cycloheximide, cells of *P. chrysogenum* produced penicillin for more than 24 h. Under this condition antibiotic formation was stimulated by the addition of low glutamine concentrations (Fig. 1). However, as can be seen in the same figure, the use of amino acid concentrations higher than 1 mM brought about a clear inhibition of antibiotic formation, proportional to the amount of glutamine present in the system. To obtain more information about the glutamine inhibition, the possibility of its reversal by the amino acid precursors of the antibiotic moiety was explored. As can be seen in Fig. 2, mycelium of the resting-cell system, previously incubated in 2 mM glutamine, was not able to produce penicillin even when supplemented with 1 mM α-aminoadipate, cysteine or valine. The effect of glutamine on δ(L-α-aminoadipyl)L-cysteine synthetase activity, first enzyme of the antibiotic formation pathway, was tested. However, as shown in Table 1, 10 mM glutamine did not show any significant effect on this activity. Other steps of the pathway have not yet been explored. Unexpectedly, glutamine inhibition was prevented by glutathione, (Table 2). Preincubation of the cells with 1 mM glutathione prevented the glutamine suppression of antibiotic formation. Under

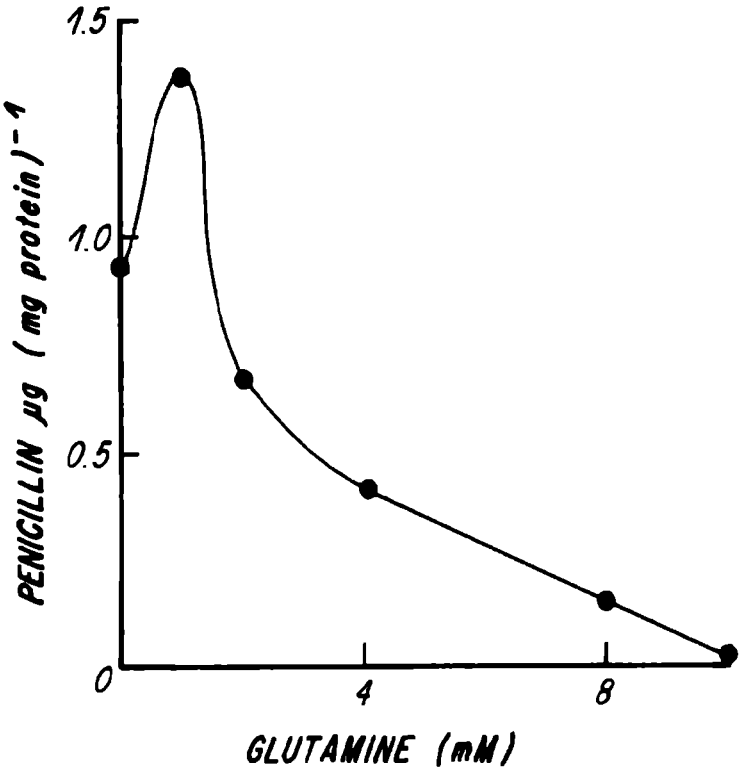

Fig. 1. Effect of different glutamine concentrations on the formation of penicillin by a resting-cell system of *P. chrysogenum*. Antibiotic production was determined after 12 h incubation at 29°C.

this condition, possible alterations in the glutamine incorporation were studied, but as shown in Fig. 3, the uptake of [^{14}C] glutamine was actually found to be higher in the presence of 1 mM glutathione. Protection against the glutamine inhibition was not given by the amino acid components of glutathione at 1 mM cocentration, singly or together (not shown).

DISCUSSION

In the resting-cell system glutamine concentrations higher than 1 mM brought about a strong inhibition of antibiotic formation with a maximum effect at 10 mM concentration. Friedrich and Demain (1977) have shown in the same microorganism that L-lysine and some of its analogues also suppressed penicillin formation. Their effect was reversed by addition of α-aminoadipate, a common precursor for the penicillin and lysine formation pathway (Demain, 1974); lysine inhibits and represses the homocitrate synthetase, first step in the lysine biosynthetic pathway (Demain, 1974; Luengo *et al.*, 1979). In contrast to the lysine effect, the glutamine inhibition was not reversed by

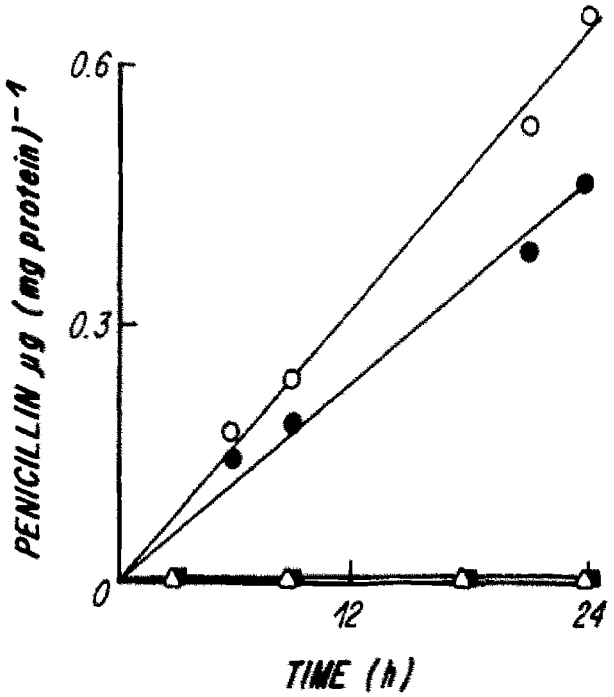

Fig. 2. Formation of penicillin by resting-cell systems previously incubated (△,■) or not (●,○) in the presence of 2 mM glutamine. After glutamine elimination, cells were resuspended in the suspension medium supplemented (△) or not (■) with 1 mM L-cysteine, L-valine and L-α-aminoadipate. Control fed (○) or not (●) with antibiotic precursors.

Table 1. *Effect of L-glutamine on δ(L-α-aminoadipyl)L-cysteine synthetase activity*[a]

Condition	Specific activity µkat Kg protein^{-1}
Control	145
L-Glutamine[b]	165

[a] The activity was carried out during 40 min at 37°C.

[b] Added to the reaction system at a final concentration of 10 mM.

addition of the amino precursors of the antibiotic moiety, and glutamine had no effect on the activity of the first enzyme in the penicillin formation pathway. This inhibition by glutamine is an effect which is probably quite unrelated to the glutamine stimulation observed with low amino acid concentrations; this view is supported by the reproducibility in the stimulatory action of the amino acid (Sanchez *et al.*, 1981), and by the fact that α-aminoadipate, cysteine and valine caused no reversion of the inhibition exerted by high glutamine concentrations.

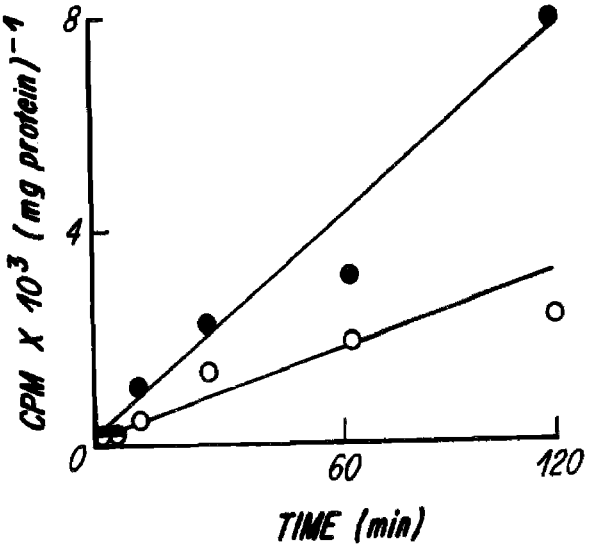

Fig. 3. Incorporation of [^{14}C] glutamine (0.6 µCi µmol^{-1} by a resting-cell system supplemented (●) or not (○) with 1 mM glutathione.

Table 2. *Formation of penicillin by resting-cell systems of P. chrysogenum*

Conditions[a]	Penicillin formation[b] µg mg protein^{-1}
Control	0.26
L-Glutamine	0.00
Control with cells preincubated in glutathione	0.25
L-Glutamine with cells preincubated in glutathione	0.21

[a] After preincubation during 1 h in the presence or not of 1 mM glutathione, the tripeptide was eliminated and the cells were resuspended in the suspension medium supplemented or not with 10 mM glutamine.

[b] Determined after 12 h incubation at 29°C.

Surprisingly, when the cells were preincubated with 1 mM glutathione, the glutamine inhibitory action was prevented. This effect does not seems to have any relationship with the uptake of glutamine (since uptake of the labelled amino acid was even higher in the presence of glutathione) and seems to be brought about by glutathione itself (since its constituent amino acid, together or separated, were not able to prevent the glutamine inhibition).

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