EFFECT OF CHLORAMPHENICOL ON ACTIVE AMINO ACID TRANSPORT

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1. Introduction

Chloramphenicol (CAP) inhibits 2-aminoisobutyric acid (AIB) influx into *Streptomyces hydrogenans* by 50%, but only after exposure to the inhibitor for at least 20 min. Inhibition of transport by inhibitors of protein synthesis has already been reported in other cell species. A widely accepted explanation of this effect is that proteins constituting the transport system turn over rapidly, so that inhibition of *de novo* synthesis will rapidly deplete these proteins. This was discussed e.g. by Elsas and Rosenberg [1], who observed inhibition of amino acid transport in kidney slices during incubation with puromycin. An alternative explanation was presented by Grenson et al. [2], who observed inhibition of amino acid transport in yeast cells by cycloheximide. These authors assumed that this inhibitor of protein synthesis acts indirectly on transport, the primary event being a release of amino acids from protein into the cellular pool, these then reduce uptake by transinhibition. Our experimental data can best be explained by assuming that some protein which is involved in carrier-mediated transport has a rapid turnover and its amount is reduced by CAP treatment so as to become rate-limiting.

2. Materials and methods

Streptomyces hydrogenans cells were cultured and harvested as previously described [3]. The cells were suspended in 0.05 M phosphate buffer, pH 7.1, to give a final cell density of 1.5 mg cell dry weight per ml suspension. The uptake at 30° was measured using labelled amino acids. [U-^{14}C] aspartic acid (6 mCi/mmole) and [1-^{14}C] 2-aminoisobutyric acid (45 mCi/mmole) were obtained from the Radiochemical Centre, Amersham. Incubations were stopped by rapid filtration of 2 ml of the suspension on a membrane filter, followed by washing of the cells on the filter with buffer. The counts per min per g dry weight (u'_c) divided by the counts per min per ml medium (a'_f) is defined as the relative uptake Ru'. Chloramphenicol was obtained from Boehringer and Soehne, Mannheim.

3. Results and discussion

When *Streptomyces hydrogenans* are incubated with CAP (50 mg/ml) for more than 20 min, an inhibition of the influx of AIB and aspartate into the cells becomes apparent. This concentration of CAP is high enough to block growth of the organism completely. Fig. 1 shows the decrease of transport activity with increasing duration of CAP treatment. The effect only becomes measurable after a lag period and after 60 min the influx of AIB and aspartate is inhibited by about 50%. Formal kinetic analysis characterises the inhibition as of the non-competitive type.

This inhibition could be explained on the assumption that a protein, essential or auxiliary to the translocation process, turns over rapidly and that its concentration after CAP treatment becomes limiting. Whether such a protein is degraded or undergoes alterations, which influence its normal function, cannot be decided.

Grenson et al. [2] discussed an alternative explanation of such transport inhibition assuming that the expansion of the intracellular free amino acid pool resulting from protein breakdown inhibits uptake

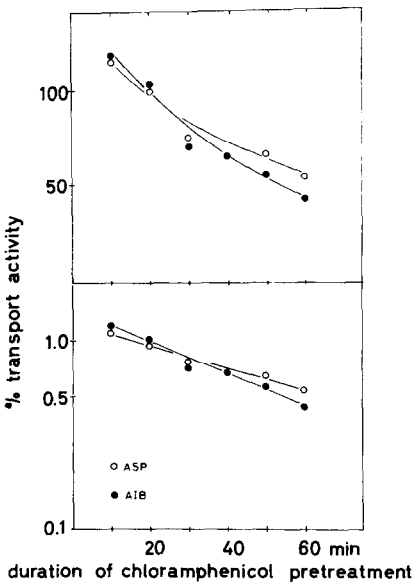

Fig. 1. Effect of chloramphenicol on active amino acid uptake in *Streptomyces hydrogenans*. Abscissa: time of incubation with 50 μg CAP/ml. Ordinate (upper figure): percentage transport activity, linear scale. Ordinate (lower figure): percentage transport activity, logarithmic scale. Each point represents the transport activity measured during a 2 min influx from [^{14}C] 2-aminoisobutyrate (0.32 mM) (•); or [^{14}C] aspartate (0.32 mM) (○). The relative uptake values of controls without CAP were taken as 100%, for AIB Ru' = 578.8 and for aspartate Ru' = 138.7.

by a specific kind of feedback control. Since the uptake of amino acids into *Streptomyces hydrogenans* is controlled by the intracellular concentration of amino acids [4], such an explanation would appear also to fit the present results.

If the rate of uptake is controlled by the level of intracellular amino acids, removal of these should abolish the inhibition of uptake by CAP. As has been shown previously [5], cold shock reversibly opens the cell membranes of *Streptomyces hydrogenans*, and low molecular weight intracellular substances are released into the medium. With this procedure the labile amino acid pool can be drastically reduced [6]. In cells pretreated for 60 min with CAP and subsequently cold-shocked, the inhibition of amino acid uptake was not abolished and the resting activity was not appreciably changed (table 1). The small reduction of inhibition observed may be due to the loss of intracellular amino acids. It should be noted that transinhibition of amino acid uptake caused by AIB-preloading can be almost completely removed by cold shock [4].

Since AIB is a strong transinhibitor of amino acid influx [4], inhibition of influx by CAP should be more pronounced in untreated cells than in cells in which uptake is maximally inhibited by AIB-preloading. In other words, preloading with AIB should not be expected to be as effective in inhibiting

Table 1

Effect of removing intracellular amino acids from CAP-treated cells by cold shock. *Streptomyces hydrogenans* were pretreated with CAP (50 μg/ml) for different time intervals or handled in the same way without inhibitor. Subsequently, the 2-min-uptake of [^{14}C] AIB (0.45 mM) was measured. After the given intervals aliquots of both suspensions were cold-shocked at −2° to remove intracellular amino acids, and the cells were resuspended in fresh medium at 30°. The AIB-influx was then measured under identical conditions.

Duration of pretreatment (min)	Ru'			
	Control suspension without inhibitor		Suspension containing 50 μg CAP/ml	
	(A)	(B)	(A)	(B)
30	275.7	324.8	130.0	131.0
50	302.2	305.2	120.1	135.9
70	324.9	318.1	91.9	120.3

(A) = before cold shock.
(B) = after cold shock.

Table 2
Uptake of AIB (0.45 mM) in phosphate buffer, pH 7.1, at 30°. Cells were treated with 50 μg CAP/ml and/or preloaded with AIB (4.3 mM). After 60 min the cells were centrifuged down and resuspended in fresh buffer. Subsequently, the influx of AIB was measured.

Pretreatment	Preloading	*Ru'*	Influx (μmoles/g 2 min)	% Transport activity
Buffer	none	201.8	90.8	100
CAP	none	87.2	39.2	43.2
Buffer	AIB	76.7	34.5	38.0
CAP	AIB	36.0	16.2	17.8

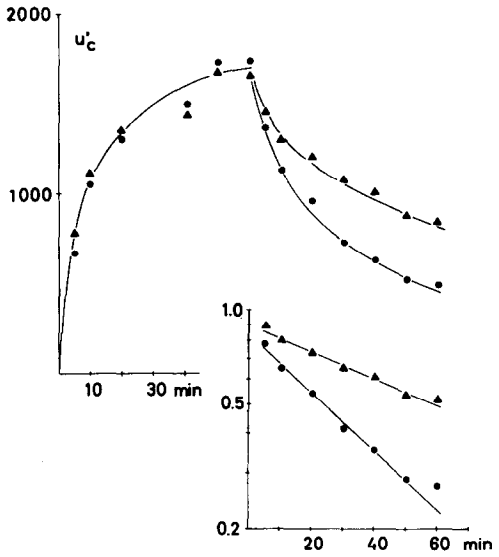

Fig. 2. Net uptake and efflux of 2-aminoisobutyrate by cells incubated in buffer with 50 μg/ml CAP (▲) or without CAP (△). First incubation: first 60 min in buffer containing 0.6 mM [^{14}C] AIB. Second incubation: second 60 min in fresh buffer containing 1.2 mM AIB. Ordinate: counts per min per mg dry weight, u'_c. Inset: logarithmic plot of efflux data.

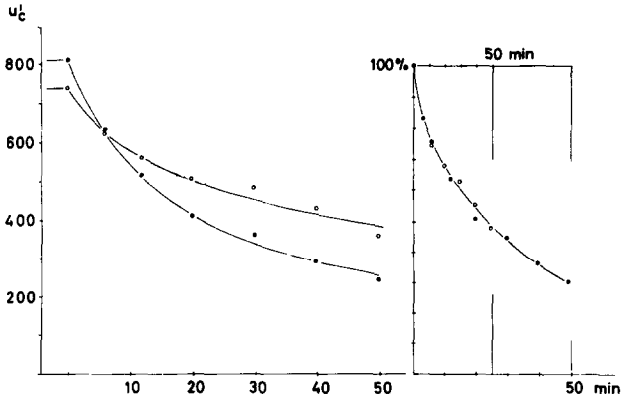

Fig. 3. Efflux of [^{14}C] 2-aminoisobutyrate from cells after preloading with this amino acid (1.2 mM) for 60 min in buffer containing chloramphenicol (○) or without (●). After preloading the cells were resuspended in fresh buffer containing 1.2 mM AIB. Left: Ordinate: u'_c = counts per min per mg dry weight; Abscissa: time in minutes.
Right: Same data as in left replotted setting the radioactivity at the start of efflux at 100% (ordinate).
Upper abscissa: time in min for CAP-treated cells (●);
Lower abscissa: time in min for control cells (○).

uptake in CAP-treated as in control cells. However, the results in table 2 show that the percentage inhibition of uptake by AIB-preloading is the same, within the limits of experimental error, whether the cells were pretreated with CAP or not. These results, like those with cold shock, are not consistent with the hypothesis that CAP acts primarily by increasing the concentration of the intracellular amino acid pool. Thus the explanation of Grenson et al. [2] for the inhibition of amino acid uptake of yeast by cycloheximide does not appear to hold for the effect of CAP on *Streptomyces* cells.

On the other hand, if the amount of an active protein directly involved in carrier-mediated transport is decreased, and assuming the test amino acid uses the same carrier for both fluxes, then inhibition by CAP should be demonstrable on both influx and efflux. The effects of CAP on the influx and efflux of AIB are shown in fig. 2. These results show that during net uptake of AIB there is no significant difference be-

tween control and CAP-treated cells but the initial velocity of efflux and the efflux coefficient are both reduced by CAP treatment. From the differing slopes of the straight lines obtained in the logarithmic plots of the efflux data (fig. 2) the increased half-life of efflux caused by CAP can be appreciated. The left hand side of fig. 3 shows an experiment in which the efflux of AIB is reduced to 50% of the control by 60 min treatment with CAP. The results are replotted on the right hand side of fig. 3 by setting the radioactivity at the beginning of efflux at 100% for both control and CAP-treated cells and by halving the time scale (upper abscissa) for the CAP-treated cells. Both curves now coincide and they can be seen to have the same shape.

From our results it may be stated that the effect of CAP cannot be explained merely as being due to trans-inhibition of active transport by intracellular amino acids released from proteins, whose resynthesis is blocked by the inhibitor. It is more likely that the concentration of a protein essential to the transport process is lowered by CAP as a consequence of its rapid turnover rate.

Acknowledgements

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