RECOVERY OF BACILLUS AMYLOLIQUEFACIENS PROTEIN SYNTHESIS FROM INHIBITION BY PACTAMYCIN

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Received April 20, 1971

SUMMARY

Pactamycin inhibits protein synthesis in B. amyloliquefaciens as expected but the cells quickly recover from this inhibition. This recovery is not due to acquisition of resistance; the results are compatible with the metabolic removal of the antibiotic.

INTRODUCTION

Pactamycin is an antibiotic believed to specifically inhibit initiation of protein synthesis[1]. In the course of other studies on extracellular enzyme synthesis by B. amyloliquefaciens it was observed that the effect of the antibiotic on protein synthesis by this organism was rapidly overcome. So far as we are aware this has not been previously observed.

EXPERIMENTAL

Cultures of B. amyloliquefaciens were grown at 30°C for 25 hr. as previously described[2].

Washed cell experiments were carried out as follows: Cells were harvested, twice washed in a salts-maltose-0.5% casamino acids medium[2] and resuspended in this medium to the original cell density. Samples (25 ml.) of the cell suspension were shaken aerobically at 30°C and at appropriate times, samples (1.0 ml.) were withdrawn, centrifuged and the supernatants assayed for α-amylase activity as previously described[3].

To determine the incorporation of L-[C^{14}]-valine into total protein, a 2 ml. sample of washed cell suspension containing 1 μC of L-[C^{14}]-valine was incubated with shaking. At various times 0.1 ml. samples were withdrawn and treated as previously described[2] except that the radioactivity on the Oxoid filters was counted by liquid scintillation.

2-[C^{14}]-Uracil incorporation into total cellular RNA was measured as previously described[2] except that liquid scintillation counting was again used; 2 ml. of washed cells were incubated with 0.6 μC of 2-[C^{14}]-uracil.

Pactamycin was a generous gift from the Upjohn Co. and from Dr. H.F. Lodish.

RESULTS AND DISCUSSION

Protein synthesis, as measured by the incorporation of L-[C^{14}]-valine into TCA precipitable material, shows a lag phase of 20 min. under the experimental conditions used (Fig. 1). This lag is possibly due to the presence initially of an intracellular pool of cold valine. The addition of pactamycin greatly inhibits protein synthesis, but the cells soon recover. The time needed for the escape from inhibition increases with increasing concentrations of pactamycin; with 0.1 μg/ml. of the antibiotic, escape occurs within 40-60 min. (Fig. 1), but with 5.0 μg/ml., full recovery is observed only after 3 hr.

The effect of pactamycin on [C^{14}]-valine incorporation observed here is specific for protein synthesis, rather than that it is exerting a transient general toxic effect on the cells. This is shown in Fig. 2 where it can be seen that pactamycin (0.5 μg/ml.) does not inhibit 2-[C^{14}]-uracil incorporation into RNA. During this time protein synthesis is almost totally inhibited. These

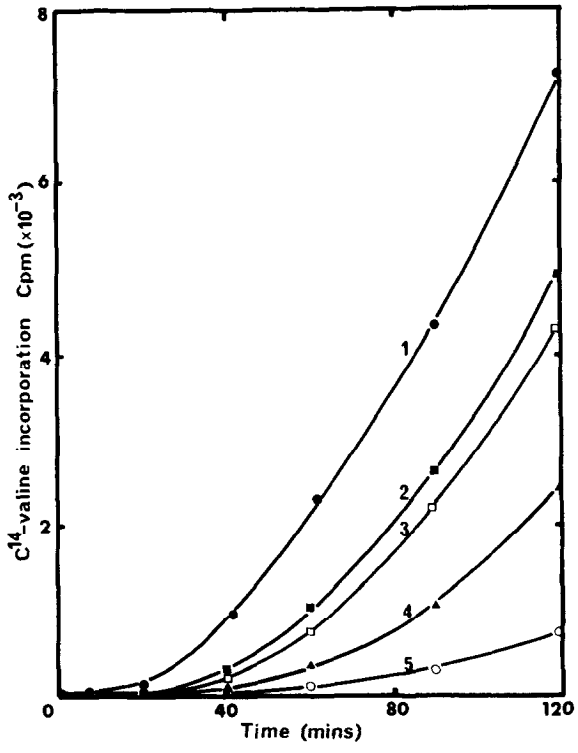

Fig. 1. Effect of pactamycin on general protein synthesis by washed cells. Pactamycin and 2-[C^{14}]-valine were both added at zero time. Curve 1 - no addition; curve 2 - 0.1 μg/ml. pactamycin; curve 3 - 0.5 μg/ml. pactamycin; curve 4 - 1.0 μg/ml. pactamycin; curve 5 - 5.0 μg/ml. pactamycin.

results are consistent with previous observations[4,5]. However, no appreciable stimulation of RNA synthesis was observed as has been previously reported in B. subtilis[4].

The possibility existed that the apparent escape from pactamycin inhibition is artefactual, in the sense that a faulty initiation process in the presence of pactamycin may allow the synthesis of nonsense protein. This is not the case. When extracellular α-amylase synthesis[3] was followed, instead of [C^{14}]-valine incorporation, the results were identical and the rate of α-amylase production was fully restored after escape.

That the escape from pactamycin inhibition does not depend on

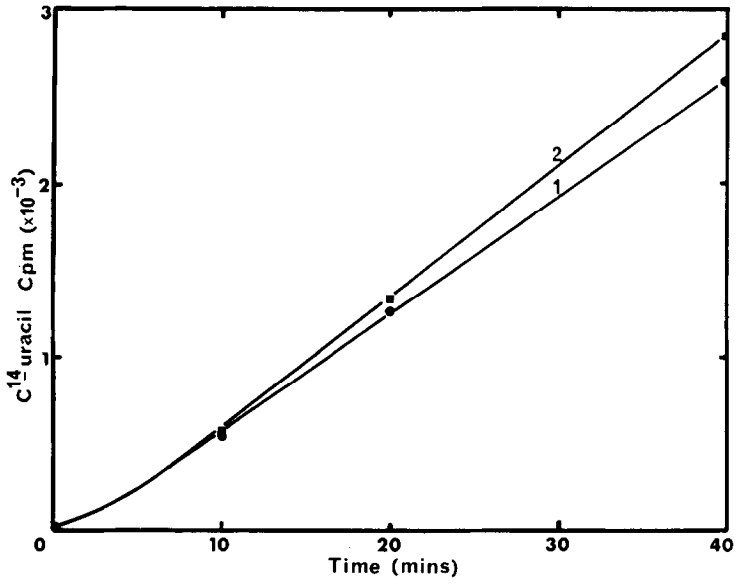

Fig. 2. Effect of pactamycin on total RNA synthesis by washed cells. Pactamycin and 2-[C^{14}]-uracil were both added at zero time. Curve 1 - no addition; curve 2 - 0.5 μg/ml. pactamycin.

the acquisition of resistance to the drug is shown in Fig. 3. When cells were pre-incubated with pactamycin (0.5 μg/ml.) for 70 min. (during which time the cells completely escape from inhibition), and then transferred to new medium containing pactamycin (0.5 μg/ml.) (Fig. 3, curve 3), the rate of incorporation of [C^{14}]-valine was identical to that of control cells pre-incubated for 70 min. without pactamycin (curve 2). As expected, both cultures were initially inhibited as compared with control cells without pactamycin. This result clearly indicates that cells, once incubated with pactamycin, are equally susceptible to a second incubation with the antibiotic.

It was thought that the most likely explanation for the escape of protein synthesis from pactamycin inhibition was that the antibiotic was progressively removed from the medium. To test this idea, cells pre-incubated without pactamycin for 70 min. were resuspended in supernatant medium from cells which had escaped from pactamycin

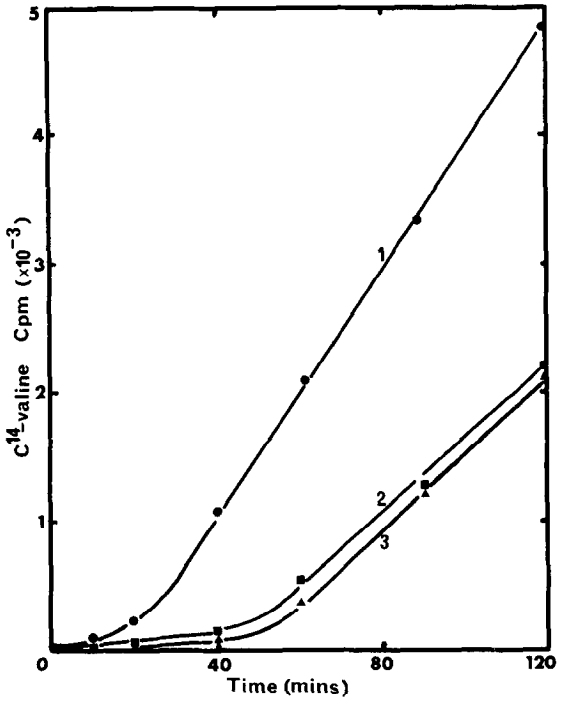

Fig. 3. Effect on protein synthesis of treating washed cells twice with pactamycin. Washed cell cultures were pre-incubated in suspending medium without pactamycin (culture 1) and with pactamycin 0.5 μg/ml. (culture 2). After 70 min., cells were centrifuged and resuspended in new suspending medium. Pactamycin (0.5 μg/ml.), where required, and L-[C^{14}]-valine (0.5 μC/ml.) were added together immediately after resuspension. Curve 1 - cells from culture 1; curve 2 - cells from culture 1 + pactamycin (0.5 μg/ml.); curve 3 - cells from culture 2 + pactamycin (0.5 μg/ml.).

inhibition (Fig. 4, curve 3). Control cells were resuspended in identical medium which had not at any stage contained pactamycin (curve 2). It can be seen that there was no inhibition of [C^{14}]-valine incorporation in the cells suspended in medium which originally contained pactamycin. This shows that the antibiotic was totally removed from the medium by the cells during the initial 70 min. pre-incubation. (The absence of a significant lag phase for curves 2 and 3 (Fig. 4) and the greater overall rate as compared with the control (curve 1) is attributed to the lower level of valine in the medium after 70 min. pre-incubation.) It appears

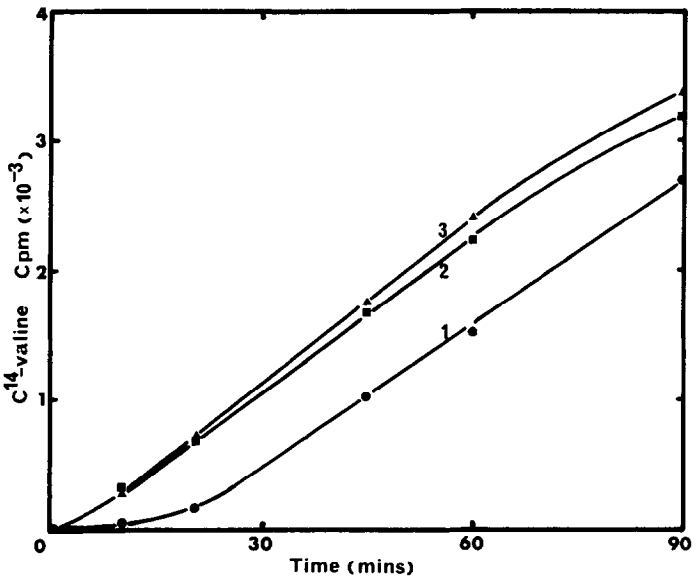

Fig. 4. Effect on protein synthesis of resuspending new cells in medium in which cells have escaped from pactamycin inhibition. Washed cell cultures were pre-incubated in suspending medium without pactamycin (culture 1) and with pactamycin (0.5 μg/ml.) (culture 2). At 70 min., culture 1 cells were centrifuged and resuspended with L-[C^{14}]-valine (0.5 μC/ml.) in the following medium. Curve 1 - fresh suspending medium; curve 2 - supernatant from culture 1; curve 3 - supernatant from culture 2.

that the mechanism responsible for the removal of pactamycin from the medium is operating at a constant rate, since the time taken for protein synthesis to escape is the same during the first and second treatments with the antibiotic.

Furthermore, this mechanism is intracellular, because a second addition of pactamycin (0.5 μg/ml.) to the supernatant of cells pre-incubated with pactamycin (0.5 μg/ml.) for 70 min. is not degraded during a further 70 min. incubation of the supernatant. Therefore, there is no extracellular product produced by the cells which destroys the pactamycin in the external medium. This is consistent with findings concerning the inactivation of nisin in several *Bacillus* species[6]. Sompolinsky *et al.*[7,8] have characterised an acquired inducible resistance to tetracyclines in *S. aureus* and

in this system, a decrease in the ability to actively accumulate the drug accounts for the resistance of these cells. Furthermore, this resistance declined when the cells were transferred to a tetracycline-free medium. However, in the present situation permeability effects seem unlikely to be involved because all the pactamycin is removed from the medium. It seems more probable that there is an enzyme system in the cells which inactivates the pactamycin. Indeed, acetylation of chloramphenicol by an acetyltransferase has been found in S. epidermidis[9]; this is an inducible enzyme which is induced by sub-inhibitory levels of chloramphenicol. Our data does not equivocally establish whether the system is inducible or non-inducible. However, the latter appears more likely in view of the fact that the pactamycin is always removed in the same time for a given concentration. It might be expected that a second treatment of the cells with pactamycin at 70 min. would result in a longer recovery time for protein synthesis, unless the induction process is complete within a very short time.

The nature of the pactamycin removing system in B. amyloliquefaciens has not been further examined.

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