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SECONDARY EFFECT OF 8-AZAGUANINE ON THE INDUCED OR CONSTITUTIVE SYNTHESIS OF PENICILLINASE IN *BACILLUS CEREUS*

by

H. CHANTRENNE
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1964

Euratom Contract No. 007-61-10 BIAB

Reprinted from
BIOCHIMICA ET BIOPHYSICA ACTA
Vol. 72, No. 1 - 1963

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Biochimica et Biophysica Acta
Elsevier Publishing Company,
Amsterdam
Printed in The Netherlands

BBA 8268

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(Received February 5th, 1963)

SUMMARY

1. During the restoration of protein synthesis caused by guanosine after inhibition by azaguanine, the differential rate of synthesis of penicillinase (penicillin amidohydrolase, EC 3.5.2.6) is much reduced.

2. This effect is observed irrespective of the state of induction, or repression, of the penicillinase-making system during the action of azaguanine.

INTRODUCTION

Previous research from this laboratory[1,2] established that 8-azaguanine disturbs the synthesis of protein in *Bacillus cereus* in two different ways. The primary effect is a general inhibition of protein synthesis which is completely expressed in 10 min; it is due to the incorporation of azaguanine into some rapidly renewed RNA[2,3], possibly messenger RNA. Secondary effects of the drug are noticed[2] when guanosine is added to the bacteria at various times after azaguanine. When guanosine is added within 15 min after azaguanine, recovery of protein synthesis is rapid and complete; recovery becomes more and more difficult if the abnormal purine is allowed to act for longer time periods. Under these conditions, restoration does not occur simultaneously for all the individual proteins. For instance, recovery of the synthesis of constitutive penicillinase (penicillin amidohydrolase EC 3.5.2.6) can lag 2 h behind that of total protein material as measured by the incorporation of [^{14}C]phenylalanine. The differential rate of penicillinase synthesis, when it begins again, is much reduced. On the contrary the synthesis of catalase recovers earlier than that of most protein material. During the period of recovery, abnormal proteins may be formed[1,4].

The purpose of the experiments reported in the present paper is to establish whether the development of these differential effects depends on the state of activity of the individual protein-making systems concerned.

Azaguanine is allowed to act before, during or after the induction of penicillinase synthesis in an inducible strain; the effects on penicillinase synthesis during restoration by guanosine are compared to those observed in a penicillinase-constitutive strain.

MATERIAL AND METHODS

Bacteria

Inducible strain: *B. cereus* NCTC 569.

Penicillinase constitutive strain: mutant isolated from the inducible strain by the method of KOGUT *et al.*[5].

Culture medium

According to POLLOCK *et al.*[6]. The casein hydrolysate used was the Difco preparation "vitamin-free casamino acid".

Chemicals

8-Azaguanine was obtained from California Corp. for Biochemical Research, guanosine from Schwarz Lab. Inc., and uniformly labelled L-[^{14}C]phenylalanine from the Radiochemical Centre, Amersham.

Experimental procedure

The bacteria were grown at 30° in well-aerated cultures until they reached an absorbancy of 0.5–0.6; they were collected by centrifugation at room temperature, washed once with water and immediately resuspended in fresh culture medium containing citrate and Tween-80 (see ref. 7) to give a suspension of absorbancy of 0.35. The suspension was distributed, in 20-ml portions, into 100-ml erlenmeyer flasks which were shaken in a thermostatic water-bath at 30°. Uniformly labelled L-[^{14}C]phenylalanine was added to each flask (2 μC and 32 μg). Further additions of 8-azaguanine or guanosine were made as indicated in the figures.

Samples, of 1 ml in volume each were mixed with 0.1 ml of a 0.01 M solution of 8-hydroxyquinoline in ethanol and kept at 0°. They were used for the determination of penicillinase activity as described previously[2]. Parallel samples of 1 ml each were mixed with 1 ml of ice-cold 10 % trichloroacetic acid solution and kept at 0°. They served for measuring the incorporation of phenylalanine into protein material, as described in a previous publication[8].

RESULTS

Previous work from this laboratory[2] has shown that the differential effects of azaguanine on the synthesis of individual proteins are easily observed with bacteria which have incorporated azaguanine for 45 or 60 min. In the experiments reported in the present paper, 8-azaguanine, at a concentration of 36 μg/ml, was allowed to act for 45–90 min before guanosine (135 μg/ml) was added. The control flasks received 8-azaguanine and guanosine simultaneously.

Restoration of penicillinase synthesis in the constitutive mutant

Fig. 1 shows the results of a typical experiment. Azaguanine was added 30 min after the addition of the labelled amino acid; the purine analogue was allowed to act for 60 min before guanosine was added. The amount of constitutive penicillinase activity is plotted against the amount of [^{14}C]phenylalanine incorporated into protein material. If the synthesis of penicillinase had been restored to the same extent as the incorporation of phenylalanine into protein material, the experimental points would

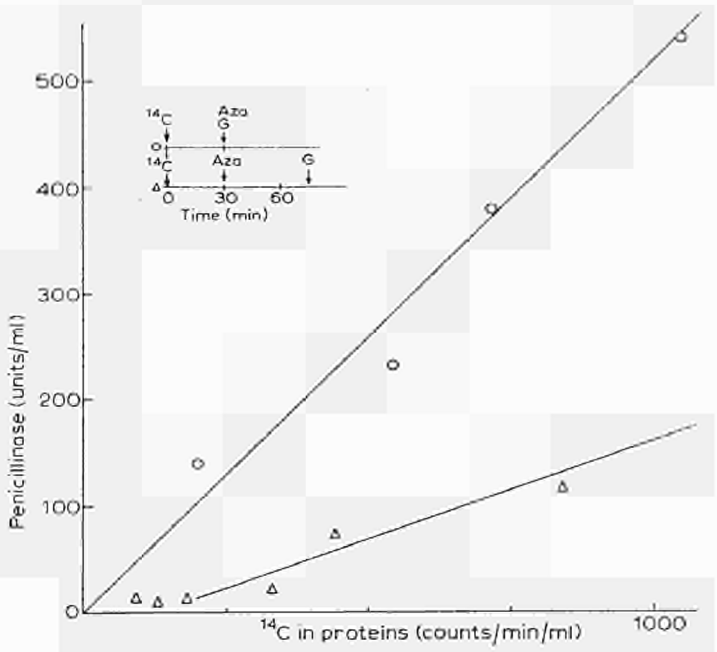

Fig. 1. Restoration of penicillinase synthesis in the constitutive mutant. Time-schedule of addition of azaguanine (Aza), and guanosine (G): as indicated in the insert. Penicillinase activity is plotted against [^{14}C]phenylalanine incorporated in protein material.

have fitted the same line as in the control. It is clear that during the period of recovery after inhibition by azaguanine the differential rate of penicillinase synthesis was reduced considerably: for a given amount of protein material made, during recovery only one third as much penicillinase activity was produced as in the control. This result confirms our previous observations[2].

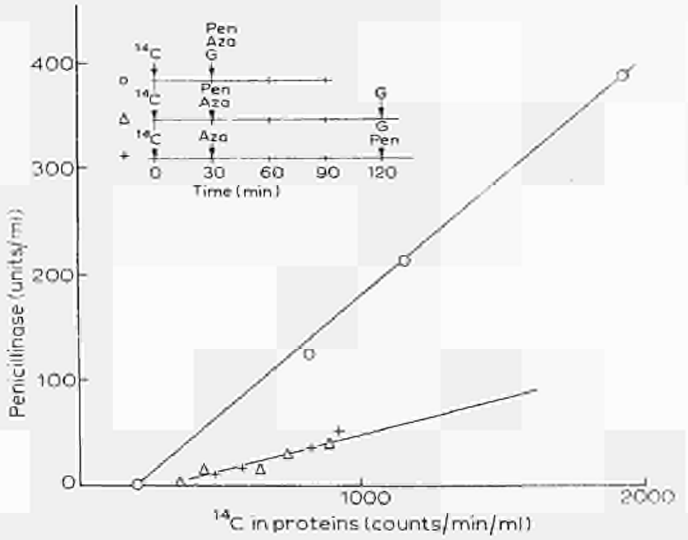

Fig. 2. Restoration of penicillinase synthesis in the inducible strain. Additions of [^{14}C]phenylalanine, azaguanine (Aza), guanosine (G) and penicillin (Pen) as indicated in the inserts.

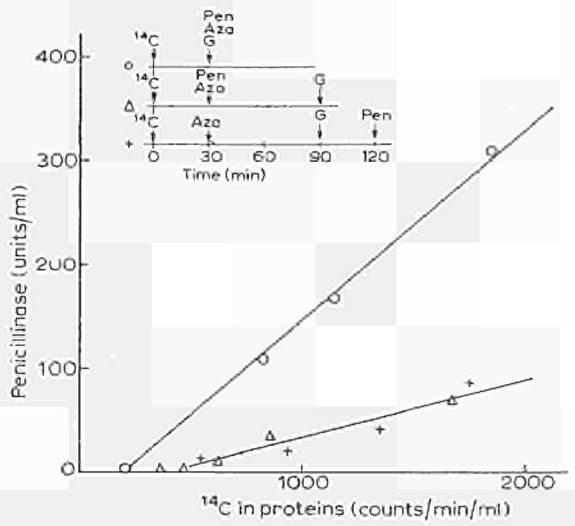

Fig. 3. Restoration of penicillinase synthesis in the inducible strain. Additions of [^{14}C]phenylalanine, azaguanine (Aza), guanosine (G) and penicillin (Pen) as indicated in the inserts.

Restoration in the inducible strain

Figs. 2 and 3 give the results of experiments similar to that in Fig. 1, except that the production of penicillinase by this strain will not occur unless penicillin is added. The inserts in each figure indicate the time schedule of addition of azaguanine which inhibits the synthesis of protein, of guanosine which restores it, and of penicillin which specifically switches on the penicillinase-making system.

In Fig. 2, penicillin was added either together with azaguanine so as to allow the analogue to act during the process of induction, or together with guanosine in order to induce when the action of the analogue was terminated.

In Fig. 3, the penicillinase-making system was induced either at the time of addition of azaguanine, or 15 min after adding guanosine, *i.e.* at a time when restoration was already well under way.

It will be noticed that in all cases, the differential rate of formation of penicillinase was much reduced in the bacteria which were recovering from azaguanine inhibition, irrespective as to whether the penicillinase-making system was in the induced state or not, during the action of the analogue.

DISCUSSION

The secondary lesions caused by azaguanine are established slowly, and they affect differently the synthesis of individual proteins. For instance the synthesis of constitutive penicillinase recovers much more slowly than that of catalase[2]. The results reported above show that these secondary lesions do affect the system which makes penicillinase regardless of the state of induction of this system during the period of incorporation of azaguanine. Whether it is maximally active as in the constitutive mutant, induced or non-induced in the inducible strain, the synthesis of penicillinase is always more severely damaged by prolonged action of azaguanine than the synthesis of the average protein material, and even than the synthesis of other enzymes like catalase[2].

The secondary lesions caused by azaguanine therefore cannot concern any RNA which is produced only when the penicillinase-making system is in the induced state. If it is assumed that the inducer causes the synthesis of messenger RNA[9], then the secondary effects of azaguanine on the synthesis of penicillinase cannot be explained by the incorporation of azaguanine into the messenger RNA which specifies penicillinase.

The damage might possibly concern some piece of the mechanism for reading or expressing the information. The decoding specificity of transfer RNA might change or be partly lost, due to the incorporation of the analogue. Azaguanine can indeed change the information of certain kinds of RNA, since it causes phenotypic reversion in several mutants of *Neurospora crassa*[10]. Another possibility is that the proper operation of the ribosomes might be affected; certain effects of streptomycin[11], which seems to act upon the ribosomes[12,13], resemble the differential damages caused by azaguanine.

These possibilities are being explored at present with cell-free preparations.

ACKNOWLEDGEMENTS

This investigation was supported by Euratom-ULB contract 016-61-10 ABIB. It was aided in part by grants from the Rockefeller Foundation, the Institut Interuniversitaire des Sciences Nucléaires and the Centre Interuniversitaire de Recherches enzymologiques.

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CDNA00509ENC